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THE CARDIO-CIRCULATORY EFFECTS IN MAN OF NEO-SYNEPHRIN

(1- α -hydroxy- β -methylamino-3-hydroxy-ethylbenzene hydrochloride)¹

By ANCEL KEYS AND ANTONIO VIOLANTE

(From the Laboratory of Physiological Hygiene, Medical School,
University of Minnesota, Minneapolis)

(Received for publication June 20, 1941)

Neo-synephrin² differs chemically from epinephrine only in the absence of the hydroxy group in the *para* position on the benzene ring. The first pharmacological studies with this substance emphasized the conclusion that the pharmacological action of neo-synephrin resembles that of epinephrine in all respects, but the potency is less and the duration of effects is longer (12, 13, 21). Inspection of the data in these papers shows, however, that the pressor effect is relatively much more prominent than the cardio-accelerator action.

The pressor action of neo-synephrin has been utilized with some success in the treatment of surgical shock (10, 11, 17), in the prevention of the hypotension of spinal anesthesia (1, 2) and in the treatment of orthostatic hypotension (6, 8). Neo-synephrin is widely used as a local vaso-constrictor and may prevent cardiac standstill in patients with a hyperactive carotid sinus reflex (18). A prominent effect of this drug in normal man is the production of marked bradycardia (14).

MATERIALS AND METHODS

The subjects ranged from 16 to 60 years of age but the majority were from 18 to 30. Thirty-nine of them were men, 9 were women. With the exception of 10 cardiac patients they were all trained as experimental subjects, so that psychic effects were at a minimum.

The studies were carried out in the morning in the basal fasting state with an absolute minimum of exciting influences. In all but a few cases the room temperature was between 75° and 80° F. and humidity was between 40 and 70 per cent. Most of the experiments were made with the subject horizontal; in the others, the subject rested in a chair designed for x-ray studies. A period of

at least one day was allowed to elapse between studies on any one subject.

The general procedure in all studies was the same. The subject rested quietly for 10 to 30 minutes and then measurements and observations were begun and continued for 10 minutes or more before the drug was administered. Observations were continued for 1 to 4 hours following the administration. In all cases blood pressure and pulse rate were measured at frequent intervals throughout the entire experimental period. The same observer measured blood pressures throughout any one experiment. Electrocardiograms were made in the majority of studies.

Roentgenkymograms (R.K.Gs.) were made in the postero-anterior position at a distance of 66 inches and an exposure time of 1.5 seconds. The R.K.Gs. were measured and analyzed for heart size and stroke output by the methods of Keys and Friedell (15, 16); in most cases the systolic and diastolic outlines were drawn by their method *B* (1940, *op. cit.*). Three or four R.K.Gs. were made in each experiment in which this method was applied.

Minute output was measured by the acetylene method of Grollman (7). In those experiments in which this method was applied the sequence was: rest 15 minutes; measurement of oxygen consumption, acetylene rebreathing; rest 10 minutes; drug administration; wait 5 to 10 minutes; measurement of oxygen consumption (8 minutes), acetylene rebreathing, final measurement of oxygen consumption.

Venous pressure was measured in the horizontal position by the direct method with citrated saline in the manometer. Circulation time (arm-to-tongue) was measured by injection of 5 ml. of a 20 per cent solution of sodium dehydrocholate ("decholin"). For this purpose the syringe needle was inserted into the vein and a minute or two allowed to elapse before the injection was started. The injection was then made as rapidly as possible and the time was measured from the start of the injection until the first sensation of the bitter taste.

Threshold for subcutaneous injection

The threshold dose of neo-synephrin to produce cardiovascular effects was determined for subcutaneous injection in 36 experiments on normal adults. In each case injection was made under the skin on the outside of the upper arm; the site

¹ This work has been supported in part by a fellowship grant from Frederick Stearns and Company. Valuable assistance was contributed by the Works Progress Administration as part of Sub-project 380, University of Minnesota Project Number 8760.

² Also known as meta- or m-sympatol and as meta-synephrin.

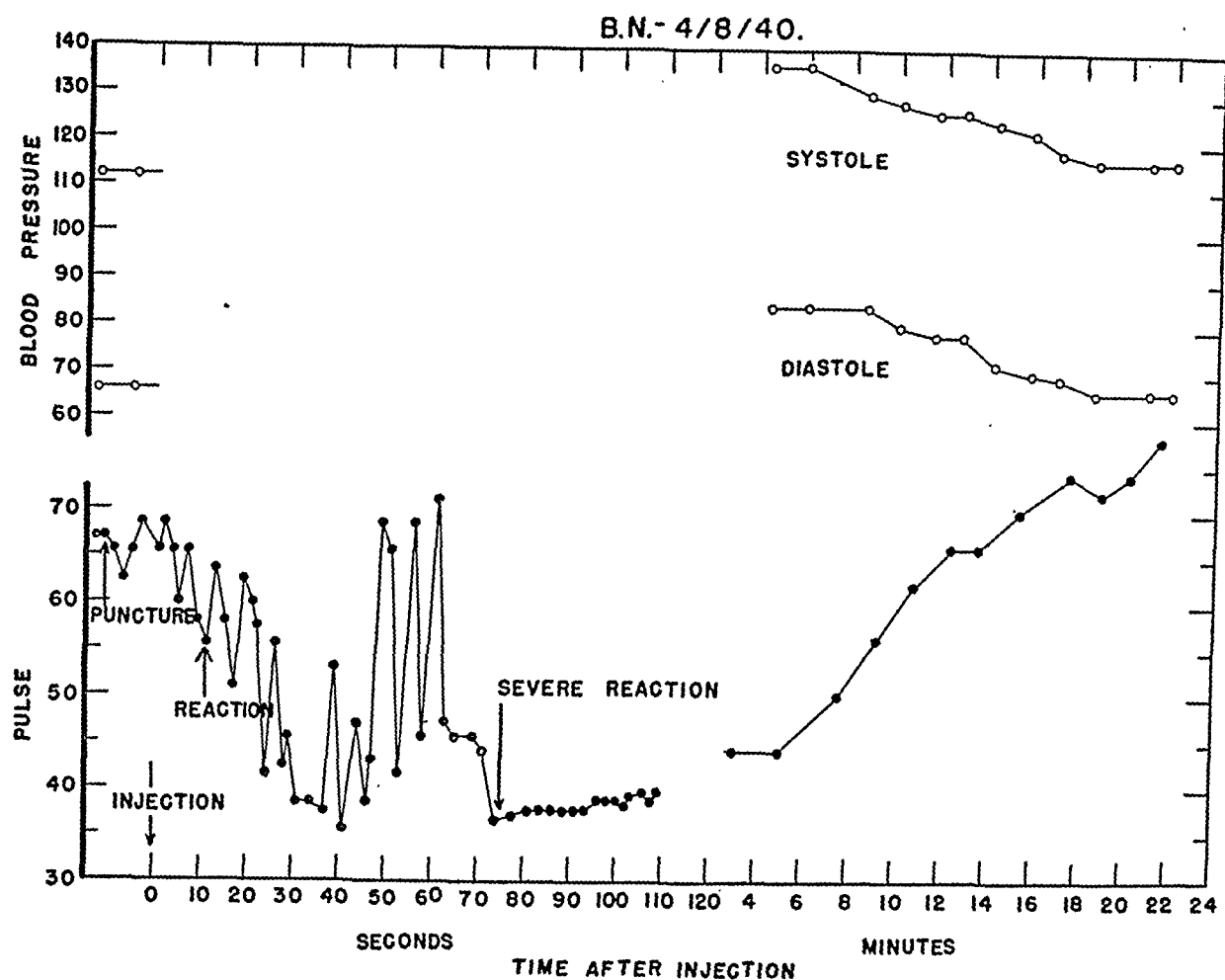


FIG. 2. BLOOD PRESSURE AND PULSE RATE CHANGES RESULTING FROM RAPID INTRAVENOUS INJECTION OF 1 MG. NEO-SYNEPHRIN IN 0.5 CC. VOLUME

"Reaction" (12 seconds after start of injection) consisted of spastic contraction of voluntary muscles, particularly those of the toes. "Severe reaction" (75 seconds) consisted of involuntary muscle twitches, jerking extremities, facial tic.

from Lead II for dosages of 4 to 6 mgm. given subcutaneously. There is always an increase in the potential of the *T* wave and there is almost always a decrease in the potential of *P*. The potential changes in Leads I and III are, in general, similar to those observed in Lead II—the potential of *P* is decreased and that of *T* is increased. In 3 cases with large doses—more than 7 mgm. neo-synephrin per square meter of body surface—the *P* wave disappeared, or became isoelectric, in all leads. In 2 instances, doses of 7 to 8 mgm. per square meter produced records indicating complete auriculo-ventricular dissociation.

One of the interesting features of the neo-synephrin bradycardia is the relative constancy of the heart rate with all but very large doses. Not only do irregularities fail to appear but the normal respiratory sinus arrhythmia is usually reduced; in

TABLE I

Electrocardiographic changes resulting from the subcutaneous injection of 4 to 6 mgm. neo-synephrin in 32 experiments

Representative normal young adults in basal rest, values before and after injection, the latter at the time of greatest bradycardia. Data from Lead II only here. Q-T interval in seconds, potentials in millivolts (corrected for calibration).

	Pulse rate	Q-T interval	Potentials		
			P	R	T
Average before.....	68	0.38	0.124	1.10	0.324
Average after.....	45	0.42	0.091	1.08	0.461
Maximum increase..	-17	0.12	0	0.15	0.47
Minimum decrease..	33	0	0.15	0.21	-0.02

5 experiments the heart rate became practically independent of respiration.

The reduction in heart rate is almost entirely the result of an extension of the diastolic pause.

The drug appears to have no effect on the conduction or spread of the nervous impulse from its normal origin.

The circulation rate

The arm-to-tongue circulation time was measured before neo-synephrin injection and again after the bradycardia was well established. With all dosages greater than 3.5 mgm. there was an increase in the circulation time, usually slight but occasionally very marked (*cf.* experiments numbered 7, 8, 10, 13).

R.K.G. measurements—heart size and output

It was suspected beforehand that, because of the increased duration of diastole, the diastolic filling of the heart would be increased with a corresponding increase in stroke output. In general, this was found to be true. An unexpected effect observed was the frequent increase in *both* diastolic and systolic size of the heart. The transverse diameter of the heart sometimes increased by more than a centimeter. It is obvious that not only does the heart fill more completely during

TABLE II

Effect of subcutaneous injection of neo-synephrin on arm-to-tongue circulation time in normal subjects as measured with sodium dehydrocholate

"Time decholin" indicates the time, in minutes, after the neo-synephrin injection when the second injection of sodium dehydrocholate was made.

Subject	Dose	Before			Time decholin	After		
		Heart rate before circulation time	Blood pressure arm	Circulation time		Heart rate before circulation time	Blood pressure arm	Circulation time
	mgm.			seconds				seconds
F.M. July 31	2.5	62	110/58	16	23'	68	114/68	16
V.M. December 5	3.5	72	120/76	14	14'	60	130/82	14
E.B. December 28	3.5	72	106/62	14	15'	52	118/76	16
L.C. December 19	5.0	58	111/70	18	16'	46	128/90	25
H.S. June 7	5.0	66	116/60	19	25'	42	144/82	23
Z.M. June 5	5.0	66	120/70	21	16'	38	154/90	23
C.S. June 8	5.0	76	108/55	22	17'	48	120/74	30
E.B. June 10	5.0	60	104/62	19	13'	42	132/90	24
Z.M. June 10	5.0	66	114/72	16	15'	44	132/84	19
L.R. June 11	5.0	66	118/64	17	13'	46	128/74	25
O.H. December 21	8.0	62	104/65	19	15'	42	139/94	21
N.S. February 1	8.0	70	118/66	19	18'	48	138/84	22
H.S. January 22	10.0	56	114/66	16	13'	34	188/100	24
Averages:		66	112/66	17.7	17'	47	136/84	21.7

diastole but that it empties less completely and there is a small amount of true dilatation.

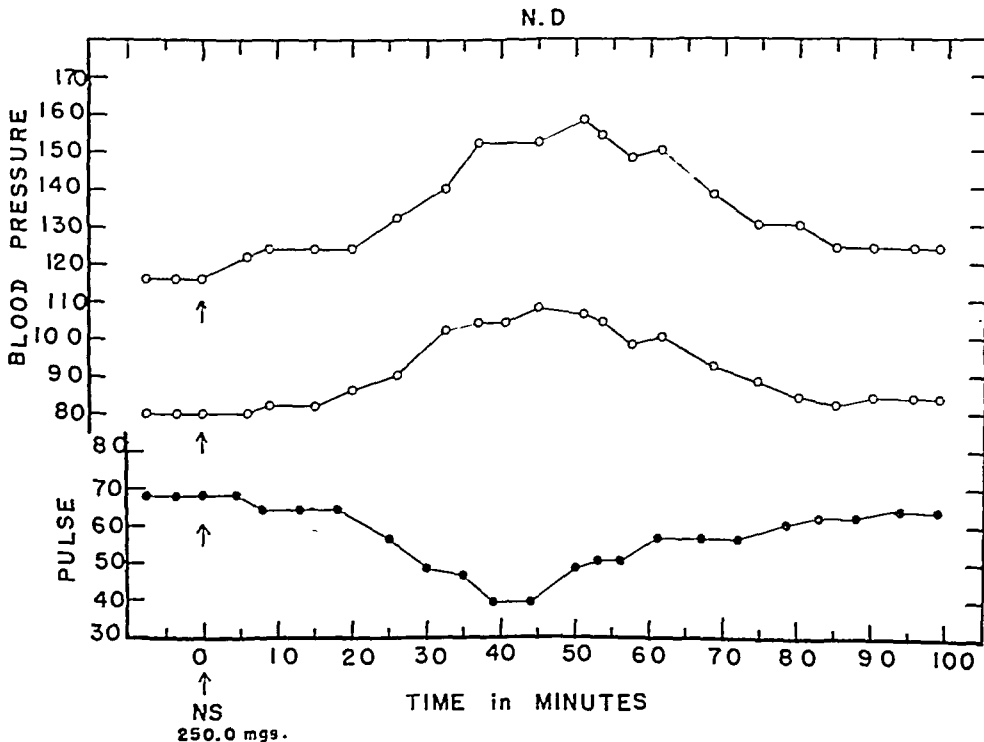


FIG. 3. BLOOD PRESSURE AND PULSE RATE CHANGES RESULTING FROM ORAL ADMINISTRATION OF 250 MG. NEO-SYNEPHRIN IN SUBJECT N. D., NORMAL MALE IN THE BASAL STATE

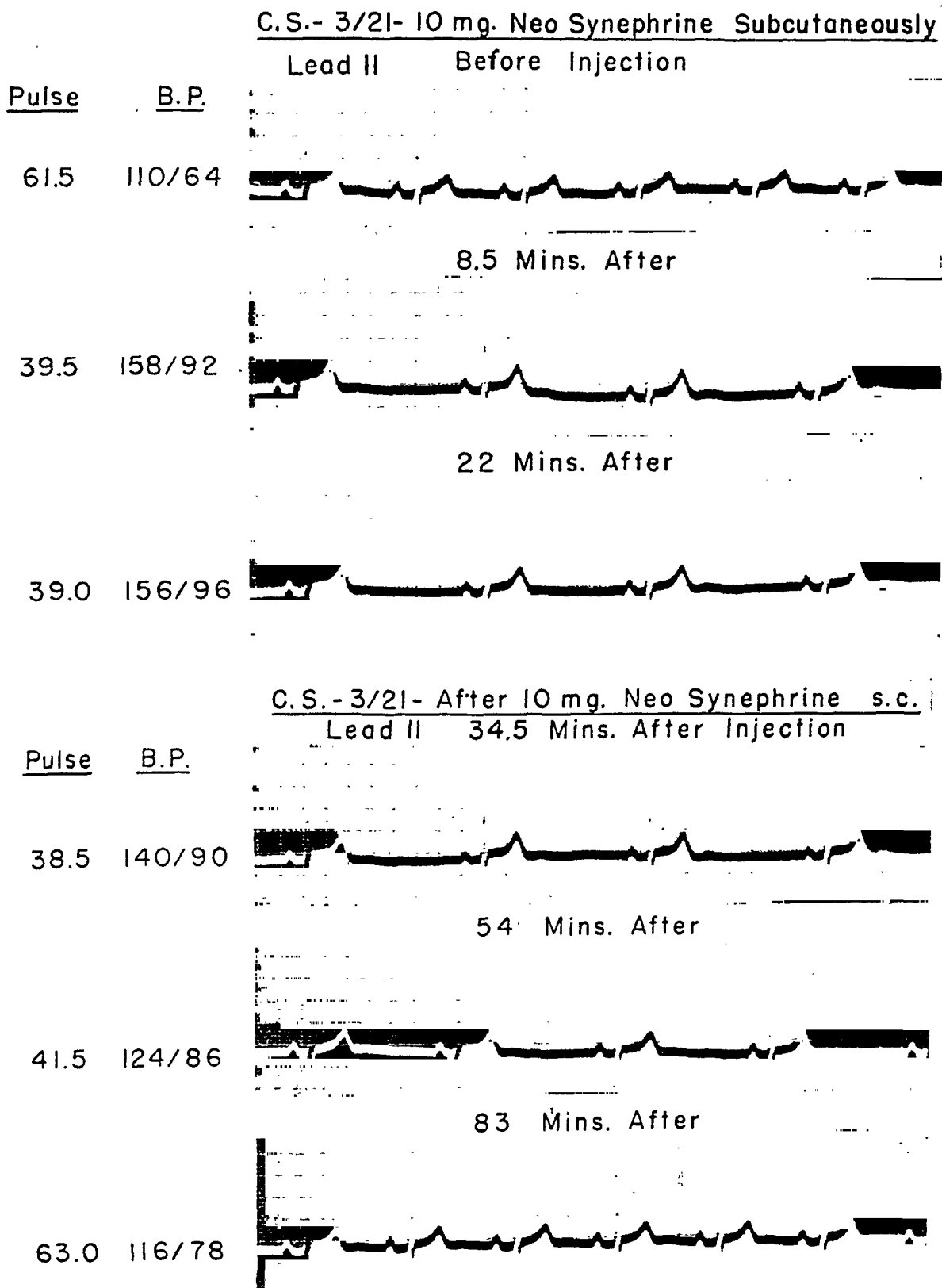


FIG. 4. ELECTROCARDIOGRAPHS (LEAD II) BEFORE AND AT INTERVALS AFTER SUBCUTANEOUS INJECTION OF 10 MG.M. NEO-SYNEPHRIN IN A NORMAL MALE

Circulation by the acetylene method

The results of 14 experiments in which the cardiac output was measured by the acetylene method are summarized in Table IV.

The results from the acetylene method are in substantial agreement with the R.K.G. measurements. There was almost invariably a large increase in stroke output, amounting on the average

TABLE III

The effect of neo-synephrin on the size and stroke of the heart as measured by roentgenkymographic methods

Seated position throughout. "Dose" in mgm. injected subcutaneously. Heart volumes and stroke outputs in cc., minute volumes in liters.

Subject	Dose	Before				After			
		Heart rate	Heart volume		Stroke volume	Minute volume	Heart rate	Heart volume	
			Diastole	Systole				Diastole	Systole
VO-1	3.5	65	656	597	76	5.1	63	691	625
LC-1	3.5	61	652	631	65	5.3	54	705	650
LC-2	3.5	62	681	629	67	5.5	63	701	646
DA	3.5	63	561	599	67	5.6	69	571	593
LE	4.0	64	497	454	55	4.6	61	521	471
BN-1	5.0	75	576	531	58	4.4	50	599	540
NS	5.0	75	734	699	70	5.3	53	809	746
EH	5.0	69	519	474	58	4.0	55	526	467
BN-2	5.0	76	359	503	69	5.2	47	591	538
DW	5.0	66	600	552	61	5.2	56	658	593
WAT	5.0	72	549	599	51	3.7	55	589	517
ZM	5.0	70	535	526	75	5.3	45	623	552
VO-2	5.0	80	597	550	60	4.8	54	610	578
LC-3	5.0	76	694	632	67	5.1	51	746	659
WIL	6.0	74	527	491	47	3.5	46	597	551
ES	10.0	82	765	724	56	4.6	55	810	753
VO-3	10.0	84	622	582	51	4.3	57	671	616
BE	10.0	75	603	555	61	4.6	42	628	565
Averages		77	612	564	62	4.8	54	645	589

TABLE IV

Measurements by the acetylene method of the minute output of the heart before injection and during neo-synephrin bradycardia

Minute volumes in liters of blood per minute, stroke outputs in cc.

Subject	Dose	Before			After		
		Heart rate	Stroke output	Minute volume	Heart rate	Stroke output	Minute volume
L.C.	December 12	3.5	81	64	5.20	54	70
GAS	December 30	3.5	82	53	4.31	67	62
L.R.*	May 6	4.0	85	59	5.05	67	58
B.N.	April 10	5.0	62	82	5.12	42	136
B.N.	April 15	5.0	66	84	5.56	42	89
DWit*	April 22	5.0	82	64	5.27	58	78
DWit*	April 24	5.0	76	62	4.75	58	91
Z.M.	June 10	5.0	77	90	6.92	45	96
VOM	December 13	5.0	79	84	6.68	75	83
L.C.	December 22	5.0	73	49	3.56	50	75
DWit	May 2	6.0	75	65	4.88	51	110
L.R.	May 20	6.0	80	48	3.84	48	127
H.S.	April 11	10.0	62	71	4.56	33	108
VOM	December 18	10.0	81	85	6.91	55	70
Averages:		76	68.8	5.19	53	89.4	4.60

* "After" determinations made during period of return of heart rate toward normal from maximum bradycardia.

to 30.0 per cent of the pre-injection value. The average increase in stroke volume was considerably greater in the measurements by the acetylene method than indicated in the experiments in which the R.K.G. method was used. This difference

may be a result of the fact that the acetylene measurements were made with the subject recumbent, while the x-ray films were always taken with the subject seated upright. We have noticed that signs of circulatory insufficiency never appeared after neo-synephrin injection when the subjects were recumbent, but in the upright seated position vertigo resulted on several occasions and once actual syncope intervened.

Effects in the atropinized subject

Ten normal subjects were used, each of whom was studied on different occasions with: (1) 4 to 10 mgm. neo-synephrin given subcutaneously, (2) 0.65 to 1.3 mgm. ($\frac{1}{100}$ to $\frac{1}{50}$ grain) atropine given subcutaneously, and (3) atropine followed by neo-synephrin. With neo-synephrin alone all of these subjects responded in the characteristic manner described earlier in this paper. Atropine alone had slight but characteristic effects on the pulse rate in all cases. An initial slight fall persisted for about 20 or 25 minutes and then was succeeded by a rise to 10 to 15 beats per minute above the basal value. Atropine alone had no significant effect on the blood pressure. As judged by the characteristic flush, dry mouth and dilated pupils, the full atropine effect was obtained in about 40 minutes and lasted 40 to 60 minutes after this.

When neo-synephrin was injected after atropinization had been established, the blood pressure in both systole and diastole immediately rose and reached higher values than those produced by neo-synephrin alone in the same subjects. The pulse rate immediately declined and then rose rapidly to 120 or more in the presence of full atropinization. With incomplete atropinization the pulse rate likewise immediately declined after injection of neo-synephrin and then rose above the basal rate and tended to remain moderately elevated. Typical results illustrating these points are summarized in Figures 5 and 6.

With a standard dosage of 5 mgm. neo-synephrin given subcutaneously, the average maximum effect in these particular subjects was an increase of 22 mm. in the systolic and 13 mm. in the diastolic blood pressure. With the same dosage in the same subjects after atropinization, the average maximum effect of neo-synephrin was an in-

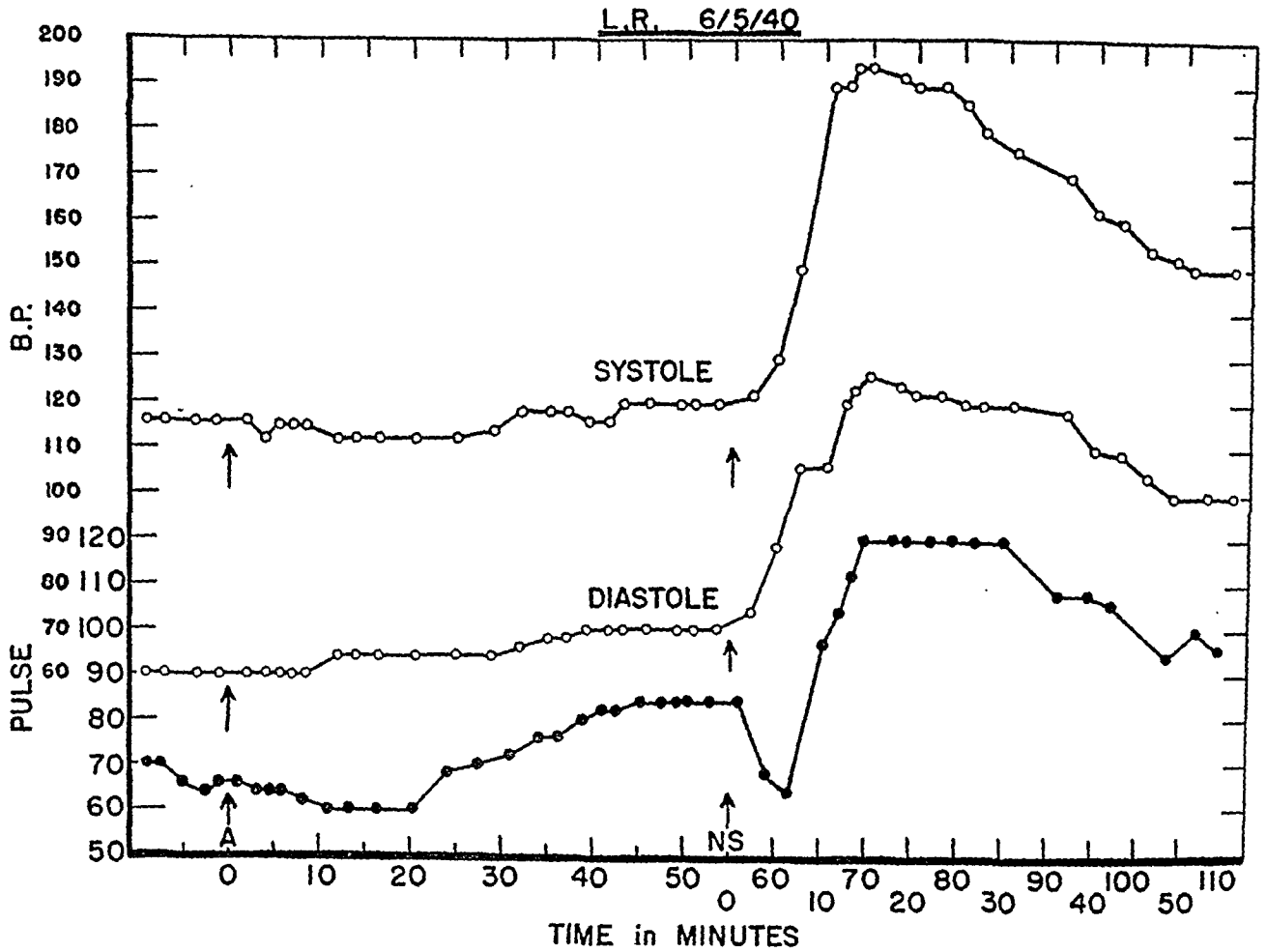


FIG. 5. EFFECT OF NEO-SYNEPHRIN IN THE FULLY ATROPINIZED NORMAL SUBJECT
At *A* 1.28 mgm. (1/50 grain) atropine subcutaneously. At *NS* 5.0 mgm. neo-synephrin subcutaneously.

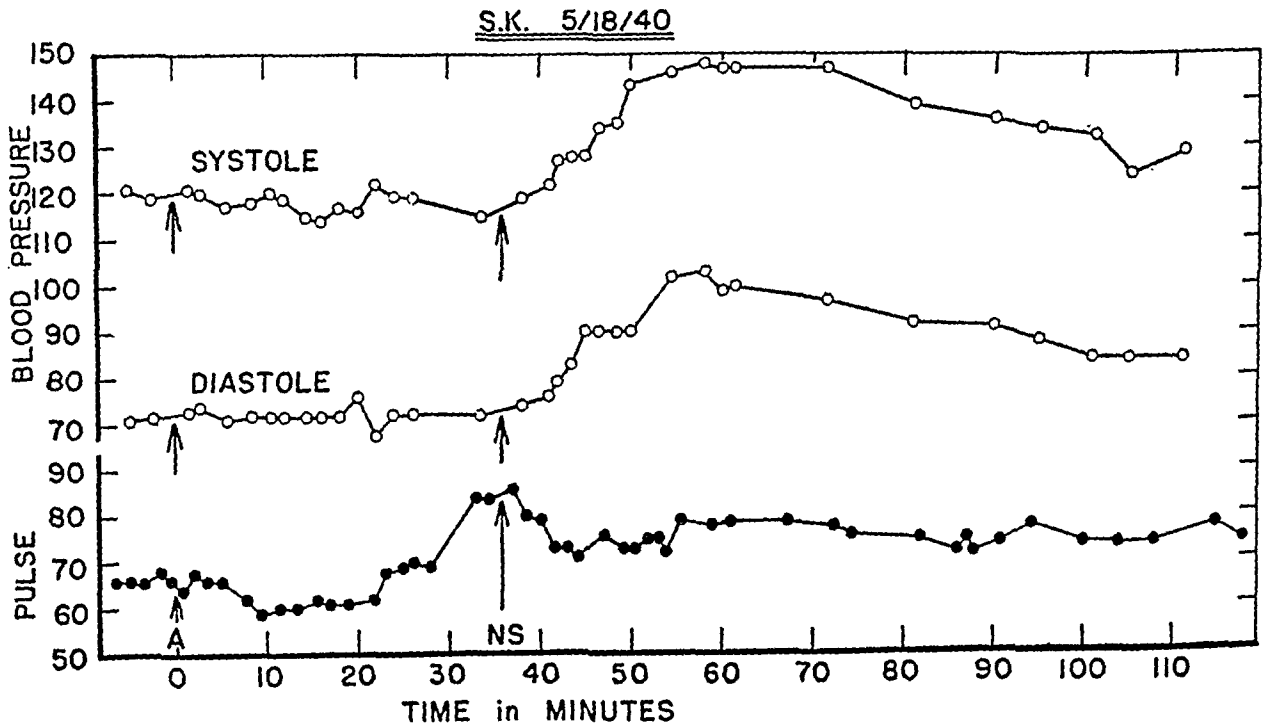


FIG. 6. EFFECT OF NEO-SYNEPHRIN IN THE PARTLY ATROPINIZED NORMAL SUBJECT
At *A* 1.28 mgm. (1/50 grain atropine subcutaneously. At *NS* 4.0 mgm. neo-synephrin subcutaneously.

crease of 62 mm. in the systolic and 52 mm. in the diastolic blood pressure. The "decholin" circulation time was measured in 5 atropinized subjects receiving 5 mgm. neo-synephrin subcutaneously. In all cases the "decholin" circulation time was shortened; the average was a reduction from 21.1 seconds to 16.9 seconds.

Patients with cardiac abnormalities

The effects of subcutaneous and intravenous injection of neo-synephrin were observed in patients presenting various types of tachycardia and of bradycardia.

In general, the results with these patients were entirely consistent with our belief that the normal effect of neo-synephrin on the heart rate operates through the vagus nerve on the sinus node. The drug was practically without effect on the heart rate in 5 cases of ventricular and supra-ventricular tachycardia. In one case of the latter, where the heart rate alternated from a rate of 60 to 70 to paroxysms of 170 lasting from 5 to 30 minutes, a total subcutaneous dosage of 25 mgm. in 50 minutes appeared to be entirely without effect except for a slight rise in blood pressure.

In another case of supra-ventricular tachycardia, the heart rate had been 180 for 12 hours before neo-synephrin was administered. Subcutaneous injection of first 5 and then 10 mgm., followed by 2 mgm. intravenously, was without effect on either blood pressure or heart rate. Further intravenous injection of 10 mgm. neo-synephrin over a period of 5 minutes increased the blood pressure by 30 mm. but still had no effect on the pulse rate.

In paroxysms of tachycardia of sinus origin, however, neo-synephrin injection has a prompt and satisfactory result. We have used the drug, in 5 mgm. subcutaneous injection, on 4 subjects who are prone to spells of sinus tachycardia but are otherwise entirely normal. On every occasion the paroxysm of tachycardia was rapidly dispelled, the heart rate dropping from a rate of 120 to 140 to 60 to 80 in 5 to 8 minutes.

According to our limited experience, more severe sinus tachycardias are likewise readily controlled with the drug. For example, Mrs. A., University of Minnesota Hospital Number 698875, who had a history of occasional very prolonged

and exhausting paroxysms of tachycardia, suddenly developed a heart rate of 160 to 180 per minute. After 16 hours the heart rate was 160, blood pressure 110/90. The E.C.G. showed the tachycardia to be of sinus origin. Subcutaneous injection of neo-synephrin, 0.2 cc., 1 per cent solution, elevated the blood pressure to 125/100 in 7 minutes with no change in heart rate. Twenty minutes after the first injection, 0.5 cc. of 1 per cent solution of neo-synephrin was injected subcutaneously. In 8 minutes the blood pressure rose to 150/105 and the pulse rate dropped abruptly to 96 and declined further to 88; in the next 5 minutes the blood pressure fell to 140/95. This patient showed no further tachycardia during her stay in the hospital.

Sinus bradycardia responds to neo-synephrin with a further decline in the pulse rate. This was noted in 3 subjects with athletic bradycardia and was particularly well shown in a case of sinus bradycardia of unknown origin in a young man with no other discernible abnormality. Before injection the heart rate was 42, blood pressure 120/62. After subcutaneous injection of 5 mgm. neo-synephrin, the heart rate fell to 34 to 36 and the blood pressure to 112/64. The E.C.G. was entirely normal at all times. The fall in blood pressure in this case is interesting though unexplained.

Subjective sensations

The administration of epinephrine in amounts sufficient to produce a pronounced pressor effect is attended by sensations of acute anxiety, cardiac oppression and throbbing blood vessels. These sensations are frequently referred to the elevated blood pressure. With a dosage as large as 10 mgm. neo-synephrin given subcutaneously, there may be a sense of cardiac oppression and fullness in the neck and chest but the feeling of anxiety is almost entirely absent. Ordinary injections of neo-synephrin—5 mgm. subcutaneously—produce no subjective sensations at all in spite of the bradycardia and hypertension.

In the atropinized subject the sudden rise of blood pressure and pulse rate following injection of neo-synephrin gives rise to a sensation of cardiac oppression and fullness in the neck and chest and may be attended with sharp pains in the head

which disappear quickly. Even in these cases, however, the typical anxiety of epinephrine injection is absent.

Neo-synephrin in large dosage—more than 8 mgm. subcutaneously—has a marked effect on the pilomoters of the skin. The hair “stands on end” and the subject usually remarks on a prickly sensation, particularly at the hair line of the forehead. In some cases “goose pimples” appear over an area of 20 to 40 sq. cm. around the site of injection.

Animal experiments

Experiments were made on dogs and rabbits for comparison with human beings and to test the interpretation of several points. Normal unanesthetized animals responded precisely like human beings to intravenous and to subcutaneous neo-synephrin—the blood pressure rose and the pulse rate fell.

The dominant rôle of the vagus in the bradycardia of neo-synephrin was shown in experiments in which the vagi were sectioned or in which the perfused isolated heart was used. In all experiments of this type the effect of neo-synephrin on the heart rate was qualitatively not to be distinguished from that of epinephrine.

DISCUSSION

By any method of administration the duration of action of neo-synephrin is longer than obtained with epinephrine. All of the subjects used in the present experiments were also tested with epinephrine; in general, the effective duration of action of neo-synephrin was from 2 to 4 times as long as that of epinephrine.

The qualitative resemblance between neo-synephrin and epinephrine is apparent in several important effects on the heart. Kuschinsky (12) reported that the coronary flow is markedly increased with neo-synephrin and later (13) demonstrated a marked dilatation of the coronaries in perfused preparations with constant blood pressure. We have also observed that neo-synephrin produces a pronounced increase in blood flow from the coronary sinus in heart-lung preparations of dogs and in isolated perfused hearts of rabbits.³ If the degree of positivity of the *T* wave of the

³ We are grateful to Dr. Gordon K. Moe for help in these experiments.

E.C.G. may be taken as an indication, we may assume a similar effect on coronary flow in man. Even in the absence of true coronary dilatation we should expect a considerable augmentation of the coronary blood flow from neo-synephrin because of the increased blood pressure and the prolongation of the diastolic period during which most of the coronary flow takes place.

Neo-synephrin has a direct stimulatory action on the denervated, isolated or vagotomized heart, both as to rate and force of contraction (*cf.* 4, 5, 22). However, the cardiac-accelerating potency is relatively much less than the pressor potency, so that even in the completely denervated heart it is possible to inject neo-synephrin at a rate which produces a pressor response without cardiac acceleration (22).

It might be suggested that neo-synephrin sensitizes or potentiates the pressure receptors in the carotid sinus and aortic arch and causes bradycardia by enhancing the reflex to hypertension. Against this view is the finding of Nathanson (18) that neo-synephrin prevents cardiac standstill produced by mechanical pressure on the carotid sinus in sensitive persons. Another significant fact is that neo-synephrin can produce definite bradycardia in man when the blood pressure is practically unaffected.

The observed increase in stroke volume resulting from neo-synephrin is accounted for by direct stimulation of the myocardium and prolongation of the period of diastolic filling of the heart. This latter effect also accounts for the increased diastolic size of the heart. The increased systolic size of the heart indicates, of course, an increase in the amount of residual blood in the heart.

The work done by the heart per beat is increased by neo-synephrin. The total work per minute is also increased on the average. With a dosage of about 5 mgm. given subcutaneously, the product of mass of blood times pressure is usually slightly increased. Moreover, since the systolic discharge period is practically unaltered and the stroke volume is increased, there must be an increase in the velocity of systolic flow in the aorta and therefore an increase in the kinetic work done.

In comparison with epinephrine and other sympathomimetic amines the action of the heart is remarkably regular with neo-synephrin. Orth *et al.* (20) compared a number of sympathomimetic

amines under cyclopropane anesthesia and found that neo-synephrin did not cause acceleration and was the least apt to produce cardiac irregularities. Cranston and Bieter (3) studied rabbits under spinal anesthesia and reported that the effective pressor dose/"toxic" dose ratio for neo-synephrin is lower than for other sympathomimetic drugs except synephrine; cardiac irregularity was the principal criterion of toxicity.

We have noted that the systolic volume of the heart is somewhat increased by neo-synephrin. This suggested the possibility that the pressure in the atria might be increased owing to less complete ventricular discharge in systole. Accordingly, direct venous pressure measurements were made in a series of experiments.

In 7 normal adults the venous pressure rose slightly after injection of neo-synephrin, being from 2 to 8 cm. (average 4.5 cm.) higher at the time of maximum effect of the drug with dosage of 5 or 6 mgm. subcutaneously. Iglauer and Altschule (9) have reported a similar but greater rise in venous pressure resulting from paredrine. They suggest that this effect in the case of paredrine, at least, is a result of "venous constriction," but we are unable to understand how peripheral venous constriction, if it occurs, can raise the venous pressure central to the valves in the veins. In the case of neo-synephrin the drug increases the output per beat of the heart but it appears that this augmentation is gained partly at the cost of a dilatation which requires a greater average venous pressure for filling the heart.

The action of neo-synephrin as a vasoconstrictor in local application is widely used. It might be thought, then, that it has a specially powerful action on the cutaneous vessels but this is not strictly true. With subcutaneous or intravenous injection, administration of neo-synephrin never produces generalized blanching and pallor like that obtained with epinephrine in equal pressor dosage.

The results of the present study indicate that neo-synephrin stimulates effectors of both sympathetic and parasympathetic nervous systems—the action of the drug is partly adrenergic and partly cholinergic. A predominance of the adrenergic action is evident in the pressor action, augmentation of the heart contraction, dilatation of the coronaries and excitation of the pilomotor system. A predominant cholinergic action on the vagus best

explains the bradycardia. Another cholinergic action is suggested in the reports of physiologically trained persons tested with neo-synephrin who state that large dosages cause definite sensations of excitation of the detrusor muscle of the bladder and inhibition of the sphincter vesicae. A relative balance of adrenergic and cholinergic effects in a number of organs is indicated in the absence of marked changes in respiration, pupil size, sweat secretion and splenic contraction. (Hemoglobin concentration is constant.)

SUMMARY

The threshold dosage of neo-synephrin in the average adult is about 2 mgm. subcutaneously, 0.4 mgm. intravenously and 50 mgm. orally. The threshold effect is a decline in pulse rate and usually a slight increase in blood pressure.

The average dosage of neo-synephrin for satisfactory pressor and cardiac effect is about 5 mgm. subcutaneously, 0.8 mgm. intravenously or 250 mgm. orally. With these dosages the pulse rate declines 15 to 35 beats per minute, the systolic blood pressure rises 15 to 40 mm., and the diastolic blood pressure rises 10 to 30 mm.

The upper limit for safe and comfortable dosage of neo-synephrin in normal adults is about 10 mgm. subcutaneously, 1.5 mgm. intravenously, and 300 mgm. per os. With rare exceptions no sensations or symptoms other than pilomotor excitation are elicited by dosages below these levels.

Neo-synephrin increases the positivity of the *T* wave, decreases that of *P*, and prolongs the diastolic pause; otherwise the E.C.G. is essentially unchanged. Cardiac irregularities, extrasystoles and escape phenomena do not occur except in rare cases with the largest doses.

Neo-synephrin produces an increase in the size of the heart in both diastole and systole. The stroke output of the heart is increased but the minute output of the heart is generally somewhat decreased. There is a slight prolongation of the circulation time and a slight increase in venous pressure. The total work of the heart is increased.

In the atropinized subject the pressor effect of neo-synephrin is augmented and tachycardia is produced. The same result is obtained in vagotomized animals and in the isolated or denervated heart.

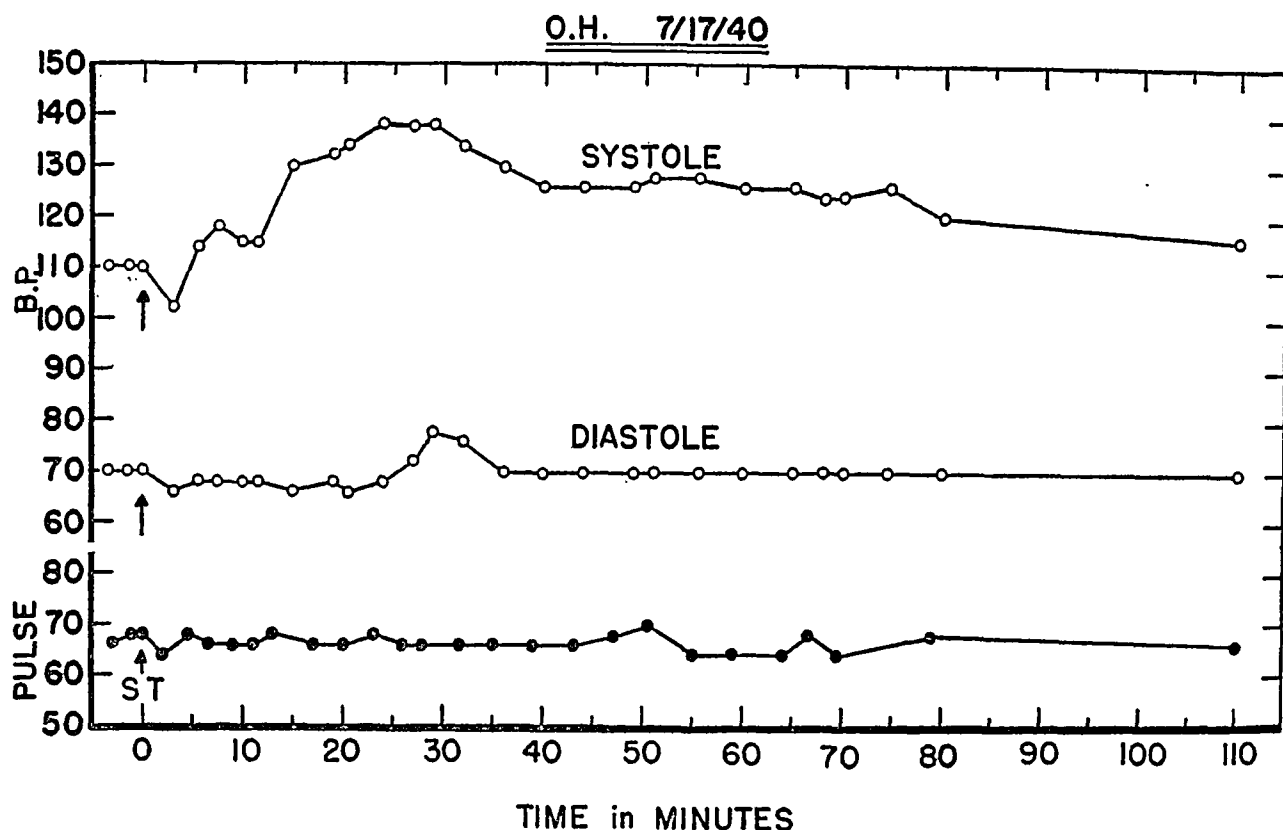


FIG. 1. BLOOD PRESSURE AND PULSE RATE FOLLOWING SUBCUTANEOUS INJECTION OF 400 MG. SYNEPHRIN TARTRATE IN 4 CC. (TWO SITES)

O. H., normal young man.

rin tartrate is shown in Figure 2. The irregularities and marked bradycardia are in sharp contrast with the results of subcutaneous injection.

The electrocardiogram

In general, synephrin tartrate in dosages up to 400 mgm. subcutaneously has no effect whatever on the electrocardiogram. In 2 instances, inversion of P_2 was produced with 400 mgm. An example of this is shown in Figure 3. In this subject, P_3 was also very slightly inverted. In contrast with epinephrine, ectopic and premature beats do not appear. Orth, *et al.* (8) noted that in dogs under cyclopropane anesthesia epinephrine produces multifocal ventricular tachycardia but that synephrin tartrate does not have such an effect in comparable (pressor) doses.

Circulation time

The circulation time (arm-to-tongue) is almost invariably shortened by synephrin tartrate in dosages of 200 to 400 mgm. given subcutaneously. The greatest reduction in circulation time tends

to appear somewhat later than the maximum pressor response. Typical results are given in Table I.

Heart size and output

The results of measurements of heart size and output by the roentgenkymographic method are shown in Table II. The stroke output of the heart is generally increased, sometimes very markedly, and the net effect of slight reduction in pulse rate and this increase in stroke is usually to produce an augmentation of the minute volume of the heart.

Measurements of the stroke output and minute volume of the heart by the acetylene method are in general agreement with the roentgenkymographic measurements but the increases produced by synephrin tartrate tend to be larger in the acetylene experiments. The explanation for this apparent quantitative discrepancy is to be found in the effect of posture (*cf.* the preceding paper). The effects of synephrin tartrate in the semi-recumbent position are illustrated in Table III.

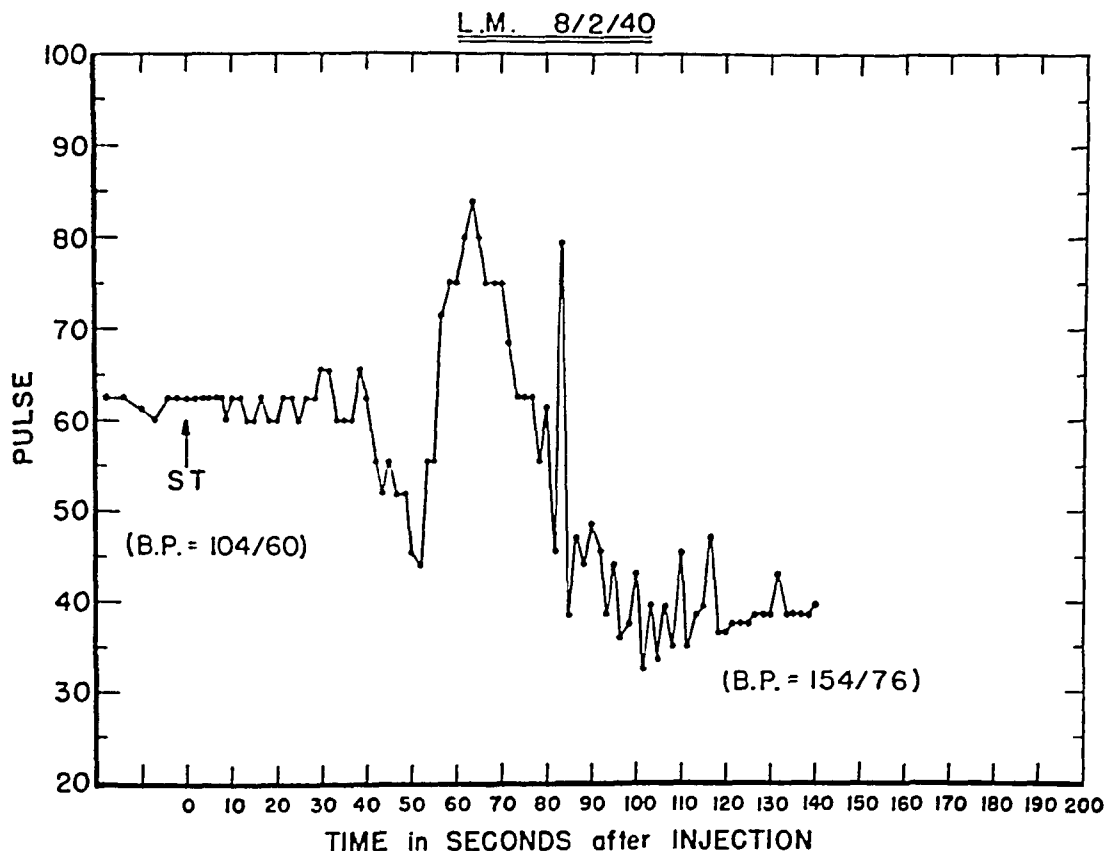


FIG. 2. INTRAVENOUS ADMINISTRATION OF 100 MG.M. OF SYNEPHRIN TARTRATE IN 5 CC. SALINE SOLUTION OVER A PERIOD OF 60 SECONDS

L. M., normal young man. Pulse rates measured by continuous E.C.G.

Effects in the atropinized subject

Synephrin tartrate injections were made in normal persons after atropine injection. Atropine dosages of 0.65 to 1.3 mgm. ($\frac{1}{400}$ to $\frac{1}{50}$ grain) were used subcutaneously; all the subjects were also separately tested with atropine and with synephrin tartrate alone. The results on pulse rate and blood pressure in a typical experiment are given in Figure 4.

In all cases the parasympathetic paralysis produced by atropine resulted in an intensification of the pressor effect of synephrin tartrate but the most marked result was on the pulse rate. After atropine, even in small doses ($\frac{1}{100}$ grain), the pulse rate was always accelerated by synephrin tartrate and the responses were qualitatively identical with those produced with epinephrine.

Subjective sensations

Like neo-synephrin, synephrin tartrate fails to produce the familiar anxiety which results when

epinephrine is given. The subjective sensations produced by synephrin tartrate, both alone and after atropine, are indistinguishable from those of neo-synephrin and, in general, are conspicuously absent with therapeutic dosages.

DISCUSSION

It is clear that both neo-synephrin and synephrin tartrate have some well-marked parasympatheticomimetic effects. From experiments with epinephrine in atropinized subjects, and in vagotomized as well as atropinized animals, a similar but smaller action on the parasympathetic system was demonstrated. Synephrin tartrate occupies an intermediate position between epinephrine and neo-synephrin with regard to the vagus-like effect on the heart rate at a given pressor dose level.

Youmans, Haney and Aumann (9) estimated the cardiac-accelerator potency of these drugs in the denervated dog heart and found epinephrine to be about 25 times as powerful as synephrin

TABLE I

Effect of subcutaneous injection of synephrin tartrate on arm-to-tongue circulation time in normal persons as measured with sodium dehydrocholate

"Time decholin" indicates the time, in minutes and seconds, after the synephrin tartrate injection when the second injection of sodium dehydrocholate was made.

Subject	Dose	Before			Time decholin	After		
		Heart rate before circulation time	Blood pressure arm	Circulation time		Heart rate before circulation time	Blood pressure arm	Circulation time
	mgm.			sec-onds				sec-onds
O.H. July 3	200	66	114/74	19	30' 44	64 62	130/64 130/68	16 16
R.E. July 9	200	70	110/60	16	29 58	62 62	128/50 128/58	15 14
L.M. July 11	250	72	110/62	19	27 37	64 74	120/62 122/70	18 19
O.H. July 17	400	78	110/70	18	25	70	142/74	15
B.E. July 16	400	58	114/58	20	33 48	60 60	142/60 136/50	16 15
O.H. July 17	400	66	110/70	22	30 53 133	66 70 64	138/78 128/70 112/70	14 14 21
L.M. July 18	400	66	110/64	20	23 45	60 62	150/66 126/64	22 22
O.H. July 18	400	78	110/70	18	25	70	142/74	15
Averages (maximum effect)		69	111/66	18.9		66	132/67	16.8

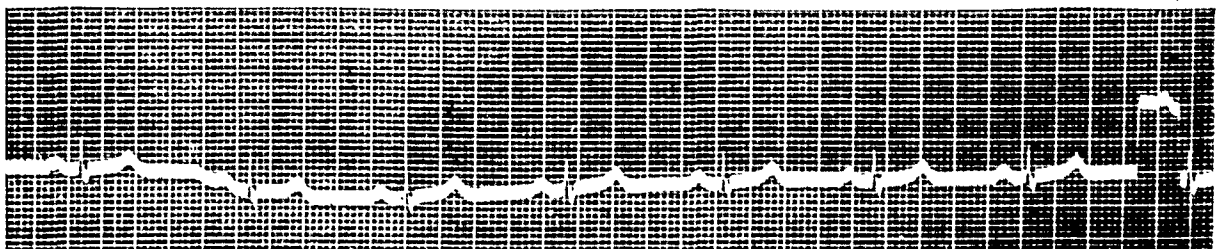
tartrate in this respect. We find the same order of difference in the fully atropinized human subject.

The early publications (*op. cit.*) on synephrin tartrate stressed the long duration of action of this drug as compared with epinephrine. We do not find any significant difference between synephrin tartrate and neo-synephrin in this respect. The low pressor potency of synephrin tartrate has mitigated against its useful application as a pressor substance. However, the toxicity of the drug is relatively very low, so that the therapeutic index—toxic dose/pressor dose—is very favorable. Cranston and Bieter (2) studied the utility of a number of pressor drugs in the prevention of the hypotension of spinal anesthesia in the rabbit. They found synephrin tartrate to be much superior to the other drugs tested in respect to the margin of safety between pressor and toxic dosage. If we consider that cardiac irregularities and "nervousness" indicate beginning toxic action, then our results in man are in full agreement that synephrin tartrate has a higher therapeutic (pressor) index than epinephrine or ephedrine.

The synephrin tartrate used by us is a racemic mixture and this is the only form at present

O.H. 8/1/40.

BEFORE.



AFTER 400 mg. S.T.

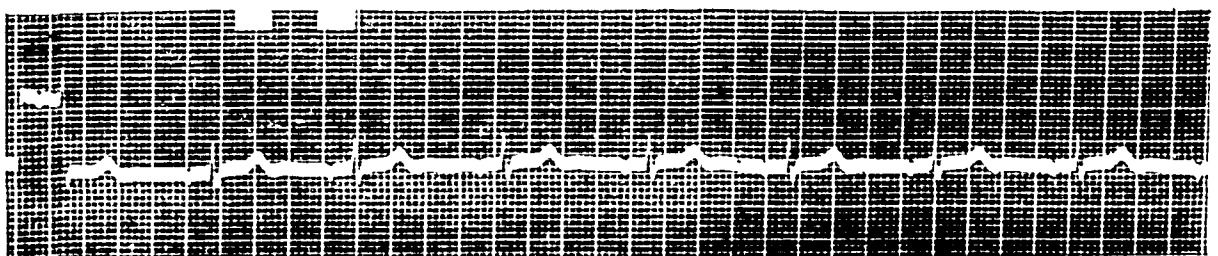


FIG. 3. INVERSION OF P_2 RESULTING FROM SUBCUTANEOUS INJECTION OF 400 MGm.

SYNEPHRIN TARTRATE

O. H., normal young man.

B.E. 8/7/40

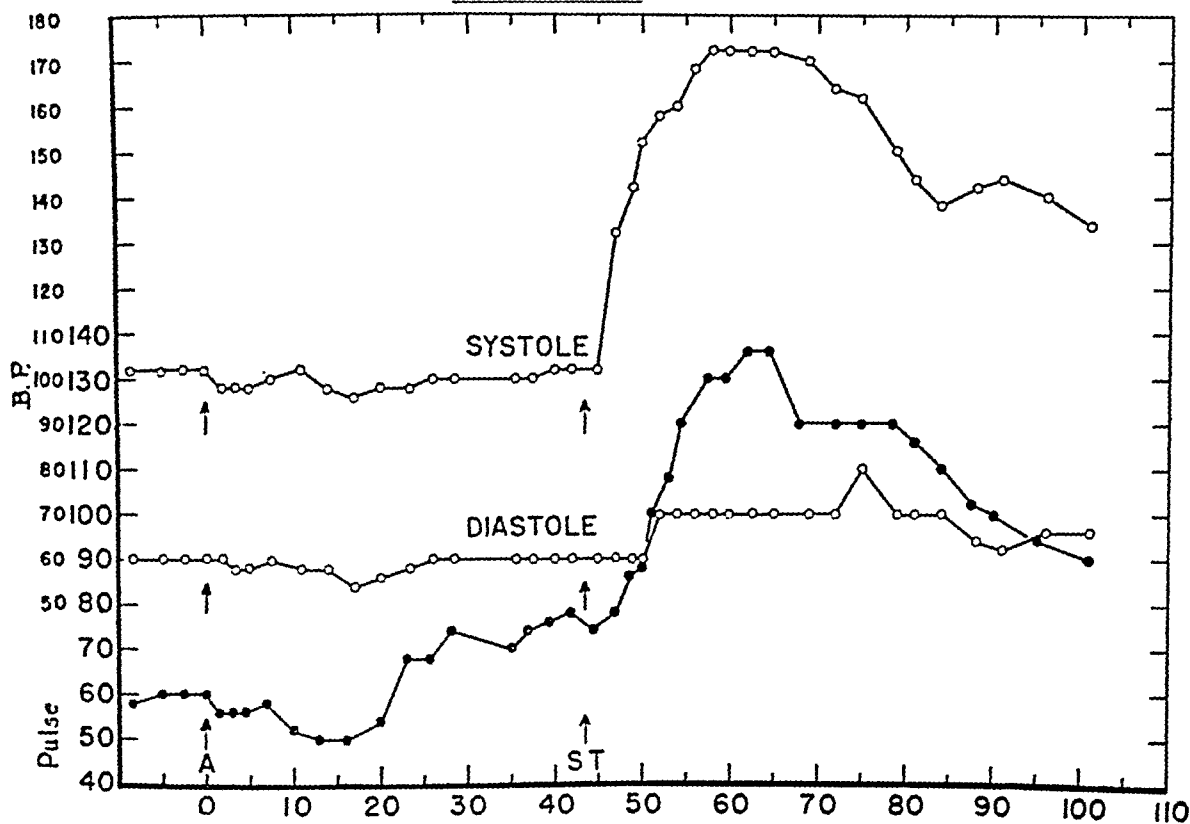


FIG. 4. EFFECT OF ATROPINE ($\frac{1}{50}$ GRAIN SUBCUTANEOUSLY) ON THE BLOOD PRESSURE AND PULSE RATE FOLLOWING SUBCUTANEOUS INJECTION OF 300 MG. SYNEPHRIN TARTRATE IN A NORMAL YOUNG MAN

TABLE II

The effect of subcutaneous injection of synephrin tartrate on the stroke output and systolic volume of the heart of normal resting young adults in the upright seated position

Each value for heart size and output is the average from measurements made on 2 roentgenkymograms. Heart volumes and stroke outputs are in cc., minute volumes in liters.

Subject	Dose	Before					Time after injection	After				
		Heart rate	Blood pressure	Heart volume	Stroke volume	Minute volume		Heart rate	Blood pressure	Heart volume	Stroke volume	Minute volume
RR 25 A	200	77	110/70	489	57	4.42	35'	68	132/70	453	53	3.65
DW 28 A	200	78	90/70	476	46	3.61	34'	71	107/70	440	63	4.77
FW 38 A	250	76	114/78	403	84	6.38	26'	64	130/74	392	90	5.69
LM 27 A	250	87	109/62	453	40	3.50	31'	74	122/70	449	56	4.14
OH 28 A	400	80	110/70	673	44	3.53	30'	73	136/74	633	66	4.78
BE 29 A	400	80	109/60	495	64	5.11	30'	75	146/58	492	70	5.23
RR 3/21	400	64	110/70	507	73	4.70	32'	60	149/74	514	86	5.18
Averages		77	107/68	499	58	4.46	31'	69	132/70	482	70	4.78

TABLE III

The effect of subcutaneous injection of 400 mgm. of synephrin tartrate on the output of the heart of normal resting young adults in the semi-recumbent position

Measurements by the acetylene method. * Values for stroke output in cc., minute volumes in liters.

Subject	Before				After			
	Heart rate	Blood pressure	Stroke volume	Minute volume	Heart rate	Blood pressure	Stroke volume	Minute volume
O.H. August 1	63	108/70	74.6	4.70	67	132/68	110.3	7.39
F.M. August 2	73	120/60	56.3	4.11	73	150/57	155.5	11.35
B.E. August 6	59	102/60	78.0	4.60	56	142/50	106.0	5.94
H.Mc. August 8	73	124/70	76.0	5.55	74	162/80	118.5	8.77
Averages	67	113/65	71.2	4.74	67.5	147/64	122.6	8.36

able. In almost all respects, except total potency, synephrin tartrate appears to warrant the claims of the German workers that it is one of the most satisfactory pressor agents yet developed.

SUMMARY

A study has been made, under controlled environmental and physiological conditions, of the

available. Since, like other related drugs, the dextro isomer is almost inert physiologically, we might predict that levo-synephrin tartrate would be a very useful drug if it could be made avail-

cardio-circulatory effects in man of racemic synephrin tartrate.

The threshold subcutaneous dosage is about 100 mgm. and the indicated therapeutic dosage for pressor action is about 400 mgm. given subcutaneously.

In normal man synephrin tartrate produces a marked rise in systolic blood pressure, a slight rise in diastolic blood pressure and a slight fall in pulse rate. With subcutaneous administration these effects are at a maximum in 10 to 30 minutes after injection and the effects persist in diminishing degree for more than an hour.

Synephrin tartrate produces a well-marked rise in stroke output of the heart and an increase in the minute volume. The arm-to-tongue circulation time is shortened.

The systolic heart size is slightly diminished with therapeutic doses of synephrin tartrate. The electrocardiogram is generally unaltered but occasionally *P* may be depressed. No irregularities in heart action have been seen in any of our studies.

It is believed that synephrin tartrate is intermediate between epinephrine and neo-synephrin in its relative sympathetico-parasympathetico-mimetic action.

BIBLIOGRAPHY

1. Chen, K. K., Wu, C.-K., and Henriksen, E., The relationship between the pharmacological action

and the chemical constitution and the configuration of the optical isomers of ephedrine and related compounds. *J. Pharmacol. and exper. Therap.*, 1929, 36, 363.

2. Cranston, E. M., and Bieter, R. N., On the prevention of the blood pressure fall during spinal anesthesia in rabbits. *J. Pharmacol. and exper. Therap.*, 1940, 68, 141.

3. Ehrismann, O., and Maloff, G., Über zweigifte der adrenalingruppe (p-oxyphenyläthanolmethylamin und sein keton). *Arch. f. exper. Path. u. Pharmacol.*, 1928, 136, 172.

4. v. Euler, U., and Liljestrand, G., The effect of adrenaline sympathol tyramine acetone and histamine on gas exchange and circulation in man. *J. Physiol. (Proc.)*, 1928, 65, 22.

5. Keys, A., and Violante, A., The cardio-circulatory effects in man of neo-synephrin. *J. Clin. Invest.*, 1941, 21, —.

6. Kuschinsky, G., Untersuchungen über Sympatol, einen adrenalinähnlichen Körper. *Arch. f. exper. Path. u. Pharmacol.*, 1930, 156, 1.

7. Lasch, F., Über die pharmacologie des sympathols, einer neuen adrenalinähnlichen substanz. (Zugleich ein beitrag zur frage der chemischen konstitution und pharmacodynamischen wirkung). *Arch. f. exper. Path. u. Pharmacol.*, 1927, 124, 231.

8. Orth, O. S., Leigh, M. D., Mellish, C. H., and Stutzman, J. W., Action of sympathomimetic amines in cyclopropane, ether and chloroform anesthesia. *J. Pharmacol. and exper. Therap.*, 1939, 67, 1.

9. Youmans, W. B., Haney, H. F., and Aumann, K. W., Relation of the groups of the adrenalin molecule to its cardio-accelerator action. *Am. J. Physiol.*, 1940, 130, 190.

THE CHANGES IN THE BLOOD PRESSURE AND IN THE RENAL BLOOD FLOW AND GLOMERULAR FILTRATION RATE OF HYPERTENSIVE PATIENTS FOLLOWING UNILATERAL NEPHRECTOMY

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In 1937 Butler (1) reported two cases of hypertension associated with unilateral kidney disease in which the hypertension was apparently abolished by the removal of the affected kidney. Since that time, there have been numerous reports (2 to 6) concerning the blood pressure changes occurring after the extirpation of a single diseased kidney in hypertensive patients. Although marked reductions in blood pressure have been reported following this type of operation, it appears that in the majority of cases the blood pressure of these patients had not returned to a normotensive level after the extirpation of the affected kidney. Indeed, Schroeder and Fish (6) in an analysis of previously published reports and a study of their own patients came to the conclusion that the efficacy of this particular surgical measure was very limited. Wilson and Byrom (7) and Friedman, Jarman and Klemperer (8) also reported that in their experimental animals the resulting hypertension from unilateral kidney derangement did not entirely disappear when the affected kidney was removed. These observers were inclined to believe that the persistence of the hypertension in their animals was related to the organic changes in the arterioles of the remaining kidney, which occurred during the prior existence of hypertension.

Since the hypertension present in individuals with unilateral kidney disease has been assumed to be due initially to this deranged kidney, it was thought advisable to study the renal hemodynamics in such hypertensive patients, both before and after the removal of the diseased kidney. In this communication, the results of such a study, together with the blood pressure changes following unilateral nephrectomy, are reported.

METHODS

Each of the five patients studied received a thorough physical and laboratory examination, and the diagnosis of unilateral kidney disease was definitively established by intravenous and retrograde urography.

The effective renal blood flow and the rate of glomerular filtration were determined by the diodrast¹ and inulin clearances, respectively. The same procedures as described in a previous report (9) were followed. A blood pressure reading was obtained at the beginning and at the end of clearance determinations and the pressure values recorded in Table I-A represent the mean of these two readings.

The clearance values of the separate kidneys were also obtained by ureteral catheterization in three of the five patients. With the exception of one patient, the clearance determinations were performed upon each patient three times—immediately before nephrectomy, 13 to 19 days after nephrectomy and again 56 to 135 days following nephrectomy. Thus the immediate and late effects of the nephrectomy upon the hemodynamics of the remaining kidney were followed. All clearance values were adjusted to 1.73 square meters of surface area.

CASES

Five hypertensive patients with unilateral kidney disease were studied. The pertinent observations in each case are detailed below.

Case 1. C. P., male, age 24 years.

History. The patient was symptomless and the hypertension was discovered on routine physical examination.

Positive findings on examination. The blood pressure before operation ranged from 130/100 to 180/120. The retinal arteries were normal in appearance. Intravenous and retrograde urography revealed an atrophic left kidney and a hypertrophied right kidney. Differential PSP revealed 22 per cent excretion of the dye from the right kidney with but a trace from the left kidney in 30 minutes. Microscopic and chemical examination of the voided urine revealed no abnormalities.

¹ The diodrast used in these experiments was given to us by the Winthrop Chemical Company.

Course. The left kidney was removed on February 14, 1941. Following the operation, the patient's pressure decreased slightly but not significantly. When examined on April 25, 1941, the blood pressure was 160/100.

Pathological study of removed kidney. The removed kidney appeared grossly hypoplastic. On microscopic examination, there was evidence of chronic inflammation. No arteriolar disease observed.

Case 2. C. S., male, age 34 years.

History. The patient was discovered to have hypertension on routine physical examination. He had no symptoms and was known to have had a normal blood pressure in 1939.

Positive findings on examination. The blood pressure before operation ranged from 140/120 to 165/125. Retinal arteries were slightly attenuated with slight degree of A-V impression. Intravenous and retrograde urography revealed a large hydronephrotic left kidney with widely dilated calyces. The right kidney appeared enlarged, but otherwise normal. Catheterized urine specimens from the separate kidneys revealed no microscopic or chemical abnormalities, and were bacteria-free.

Course. The patient was first operated upon February 20, 1941, at which time a leaf of redundant pelvic mucosa was found obstructing the ureter at its junction with the kidney pelvis. The obstruction was relieved, and the dilated pelvis was reduced in size by partial resection. Despite the relief of the hydronephrosis, the hypertension persisted. The patient was again operated upon on May 28, 1941, and the left kidney was removed. The patient's blood pressure decreased following the nephrectomy and has remained reduced. When examined on July 15, 1941, the blood pressure was 130/90. The renal clearance determinations performed upon this patient were done before and after the second operation.

Pathological study of removed kidney. The removed kidney weighed 175 grams. There was some thinning of the cortex, and the pelvis and calyces were markedly enlarged. Microscopic examination of the kidney revealed areas of chronic inflammation, but the glomeruli were normal in appearance. The smaller arteries and arterioles also were normal in appearance. Because this kidney was subjected to prior surgical measures the evidences of present infection were probably due to the nephrostomy drainage.

Case 3. J. G., male, age 18 years.

History. The patient had observed increasing breathlessness on exertion for one year.

Positive findings on examination. The blood pressure before operation ranged from 180/114 to 258/154. There was slight attenuation of retinal arteries but otherwise there were no abnormalities. Intravenous and retrograde urography revealed a normal right kidney, but a small and atrophic left kidney. Differential PSP revealed 22 per cent excretion of the dye from the right kidney and 8 per cent excretion from the left kidney in 30 minutes. Catheterized urine specimens from the separate kidneys revealed no microscopic or chemical abnormalities and were bacteria-free.

Course. The patient was operated upon March 11, 1941, and an atrophic kidney was removed. Immediately following the operation, the blood pressure fell from 208/140 to 120/70, but at the end of one week the pressure rose to 160/90. In the succeeding months it has ranged from 130/80 to 160/100. When last examined on July 26, 1941, the blood pressure was 160/110.

Pathological study of removed kidney. The kidney weighed 40 grams. The cortex was markedly distorted by old areas of scarring in which obliterated tubules, arteries and glomeruli could still be identified. Throughout the medulla and the cortex, small collections of plasma cells and lymphocytes were seen. The smaller arteries and arterioles were seen to be the seat of marked arteriosclerosis, consisting chiefly of subintimal hyalinization with partial obliteration of the vessel lumen.

Case 4. C. B., female, age 34 years.

History. Patient was operated upon in August, 1939, at which time a calculus was removed from the pelvis of the left kidney. At that time she had a normal blood pressure, but when examined again in January, 1940, it was observed that her blood pressure was 210/130. Since that time the pressure has remained consistently elevated.

Positive findings on examination. The blood pressure before operation ranged from 170/95 to 220/110. The retinal arteries were attenuated with moderate A-V impression. Intravenous and retrograde urography revealed a normal right kidney, but the upper calyces of the left kidney appeared dilated. Catheterized urine specimens from the separate kidneys revealed no microscopic or chemical abnormalities.

Course. The patient was operated upon April 9, 1941, at which time it was observed that the left kidney was surrounded by a dense scar capsule. Following the operation, the blood pressure decreased to 120/75 and in the succeeding months, it has increased slightly. When last examined on June 23, 1941, the blood pressure was 150/90.

Pathological study of removed kidney. The kidney weighed 124 grams. The cortex had many depressed, irregular areas covered by scar tissue. On microscopic examination, many areas of the kidney were seen to be filled with lymphocytes and atrophic tubules. The glomeruli appeared normal and there was no evidence of arteriolar sclerosis.

Case 5. M. F., male, age 34 years.

History. For the past several years the patient had observed increasing dyspnea on exertion. The duration of the hypertension could not be ascertained but in 1936 the patient passed a small renal calculus.

Positive findings on examination. The blood pressure before operation ranged from 195/135 to 250/150. The retinal arteries were tortuous and irregular with marked A-V impression. Intravenous and retrograde urography revealed a normal appearing right kidney but a calculus was observed in the pelvis of the left kidney which appeared to obstruct the flow of urine at the uretero-pelvic orifice. The pelvis and calyces of the left kidney were also markedly dilated. An Addis count of a 24-hour urine collection showed 100,000 casts, 71 million red blood cells and 13 million white blood cells.

Course. The patient was operated upon June 20, 1941, and the left kidney was removed. The blood pressure fell to 130/90 immediately following the operation, but on June 28, 1941, it had again increased to 160/110. Since then, the pressure has continued to remain somewhat below the pre-operative range but it is questionable whether the reduction is significant. When last examined on August 15, 1941, the blood pressure was 185/120.

Pathological study of the removed kidney. Microscopic examination of the removed kidney revealed small foci of lymphocytes and plasma cells diffusely distributed throughout the medulla and cortex. There was some obliteration of glomeruli observed. There was also severe involvement of both the smaller arteries and arterioles consisting of subintimal hyalinization, with marked encroachment upon the vessel lumen. The smaller arteries also showed considerable hyperplasia of the media.

RESULTS

A. The renal clearance determinations before unilateral nephrectomy

As Table I-A indicates, there was considerable variation in the blood pressure level of each pa-

tient as determined during his hospital stay before operation. At the time of the clearance determinations the average blood pressure of the entire series was 173/117 (range: 150/100 to 220/135).

The total effective renal blood flow (both kidneys) was found to vary considerably in the five patients studied (Table I-A). Thus, in Cases 1, 2, 3 and 5 there was a diminution in the effective renal blood flow, the values being, respectively, 720, 522, 665 and 675 cc. per minute, whereas in Case 4, the flow was 1073 cc. per minute, a value within the normal limits. In general, it appeared that the diminution in the flow was related to the severity of the hypertension (10) rather than to the specific pathology of the kidney. The renal blood flow determinations of the separate kidneys in three of the five patients (Table I-B) indicated that the blood flow in the apparently normal kidney was greater than in the diseased kidney.

The total inulin clearance (both kidneys) also was found to vary markedly (Table I-A). Thus,

TABLE I

A. Total renal clearance determinations before and after unilateral nephrectomy

Patient	Age	Before unilateral nephrectomy					After unilateral nephrectomy (13-19 days)						After unilateral nephrectomy (56-135 days)					
		Effective renal blood flow	Renal inulin clearance	Filtration fraction	Blood pressure range	Blood pressure at test	Effective renal blood flow	Renal inulin clearance	Filtration fraction	Blood pressure range	Blood pressure at test	Days post-nephrectomy	Effective renal blood flow	Renal inulin clearance	Filtration fraction	Blood pressure range	Blood pressure at test	Days post-nephrectomy
		cc. per minute	cc. per minute	per cent	mm. Hg	mm. Hg	cc. per minute	cc. per minute	per cent	mm. Hg	mm. Hg		cc. per minute	cc. per minute	per cent	mm. Hg	mm. Hg	
(1) F.P.	24	720	83.0	21.9	130/100-180/120	150/100							965	133.0	20.9	142/98 -160/100	160/100	70
(2) C.S.	34	522	118.0	31.8	145/120-165/125	155/120	590	74.5	17.8	120/80 -140/95	130/90	19	588	97.3	22.4	120/78 -130/90	128/90	56
(3) J.G.	18	665	114.0	31.3	180/114-258/154	220/135	1040	90.0	14.8	120/80 -160/100	150/90	14	875	75.0	15.6	130/80 -160/110	160/110	135
(4) G.B.*	34	1073	78.0	14.1	170/95 -220/110	170/95	1040	63.5	10.1	120/75 -130/90	130/85	13	1025	103.8	18.1	125/75 -150/90	150/90	74
(5) M.F.	34	675	132.0	28.0	195/135-250/150	195/135	381	68.7	24.0	170/105-200/120	170/120	19	422	76.0	27.6	185/120-200/130	185/120	57

* Female.

B. Renal clearance determinations of each kidney before unilateral nephrectomy

Patient	Diseased kidney			Normal kidney		
	Effective renal blood flow	Renal inulin clearance	Filtration fraction	Effective renal blood flow	Renal inulin clearance	Filtration fraction
	cc. per minute	cc. per minute	per cent	cc. per minute	cc. per minute	per cent
(1) F.P.	14	6.44	46	638	76.7	21.9
(4) C.B.	414	24.20	10	659	67.2	17.6
(5) M.F.	246			334		

in Cases 1 and 4, the inulin clearance was considerably diminished, being 83.0 and 78.0 cc. per minute, respectively. However, in Cases 2, 3 and 5, the clearance values were within the limits of normal, being 118, 114, and 132.0 cc. per minute, respectively. The clearance determinations of the separate kidneys in Cases 1 and 2 (Table I-B) indicate that, although the inulin clearance was greater than normal in the unaffected kidney, it was reduced in the diseased kidney.

The filtration fraction was high in Cases 2, 3 and 5, being 31.8, 31.3 and 28 per cent, respectively. In Case 1 the filtration fraction was 21.9 per cent and in Case 4, it was but 14.1 per cent. The reduction in the inulin clearance of these last two cases accounts for the lower filtration fraction values.

B. The renal clearance determinations after unilateral nephrectomy

It is apparent from Table I-A that, in the four patients studied immediately after nephrectomy (13 to 19 days) and while they were still hospitalized, there was a reduction in blood pressure. It was significantly reduced in three cases (Cases 2, 3, 4) and the range of pressure was slightly reduced in the fourth case (Case 5). However, it should be emphasized that all were potentially or actually hypertensive. In the succeeding weeks and months little change could be detected in the pressure values of these four patients. The remaining Case (Case 1), when followed later, showed no significant reduction in his pressure as compared to the pre-operative level. Thus, in this series, although four of the five patients had a reduction in blood pressure after operation, only in three cases was it marked. Finally, none had a normal blood pressure when last examined, although Cases 2 and 4 were observed at times to have had a normal blood pressure.

The renal blood flow was studied in four of the five patients (Table I-A) after operation (13 to 19 days). It is of interest that, in those cases showing marked reduction in hypertension, the renal blood flow remained essentially the same or increased. Thus in Cases 2, 3 and 4, the renal blood flow was, respectively, 522, 665 and 1073 cc. per minute before and 590, 1040 and 1040 cc. per minute after the extirpation of the diseased kid-

ney. However, in Case 5, the renal blood flow was 675 cc. per minute before, and 381 cc. per minute after operation. In this latter case, there was also little reduction in the blood pressure. When the blood flow determinations were repeated later (56 to 135 days) in these same four patients, it was found that comparatively little change had occurred in any of them (Table I-A). The remaining case (Case 1), when examined, had a renal blood flow of 965 cc. per minute, representing an increase over the pre-nephrectomy value of 720 cc. per minute. The observation that the renal blood flow in one remaining kidney equalled or exceeded the flow of both kidneys before nephrectomy in four out of five of these cases suggests that the remaining kidney, after nephrectomy, increased its flow. The pre-nephrectomy determinations of the flow of single normal kidneys in Cases 1, 4 and 5 (Table I-B), and a comparison of these determinations with those taken after the nephrectomy, indicate without much question that the flow had actually increased in the remaining kidney. For in Cases 1, 4 and 5, the renal blood flow was 638, 659 and 334 cc. per minute, respectively, before, and 965, 1025 and 422 cc. per minute, respectively, after the nephrectomy. Finally, it should be emphasized that, despite the fact that the last renal blood flow determinations upon Cases 1, 3 and 4 indicate that there was no renal ischemia (even if it is assumed that the remaining kidney had hypertrophied to almost twice normal size), these patients were hypertensive at the time of the last clearance examination.

In each of the four cases studied immediately after the nephrectomy (13 to 19 days), there was a reduction in the inulin clearance (Table I-A). Before operation the clearances varied from 78.0 to 132.0 cc. per minute; after operation, from 63.5 to 90.0 cc. per minute. When these four patients were re-examined later (56 to 135 days), it was found that the clearance had increased in Cases 2, 4 and 5, and decreased in Case 3. In the remaining patient (Case 1), the inulin clearance was 133.0 cc. per minute, a value much higher than that observed prior to the removal of the affected kidney. Examination of the data in Table I-B indicates that the remaining kidney actually had increased its ability to excrete inulin.

The maintenance of a relatively unchanged or increased renal blood flow and a diminution in the inulin clearance in Cases 2, 3 and 4 immediately after nephrectomy (13 to 19 days) effected a reduction in the filtration fraction. In Cases 2 and 3, the reduction was quite marked (Table I-A), but in Case 4, because of the relatively small change in the inulin clearance, there was only a decrease of 4 per cent in the filtration fraction. In Case 5, where not only the inulin clearance but also the renal blood flow decreased after operation, there was also a decrease of 4 per cent in the filtration fraction. On re-examination later (56 to 135 days), however, the filtration fraction was observed to have increased in Cases 2, 3, 4 and 5. In the remaining case (Case 1), the filtration fraction was 20.9 per cent, a value slightly lower than the one found before nephrectomy.

DISCUSSION

In a previous study of hypertensive patients without unilateral kidney disease (10), it was found that there was usually, but not always, a reduction in renal blood flow, a relatively normal glomerular filtration rate and a high filtration fraction. In the present series, the renal blood flow also was reduced in four of the five patients, but the inulin clearance was reduced in two, with a concomitantly lower filtration fraction. Because of this variability it is apparent that the diodrast and inulin clearance tests will not afford a positive clue as to the presence or absence of unilateral kidney disease, unless the kidneys are evaluated separately. If the latter is done, there will probably be a diminution in the diodrast and inulin clearances in the diseased kidney as compared to the clearances obtained from the opposite kidney.

The removal of the diseased kidney in the five patients studied resulted in an increase in the blood flow and in the glomerular filtration rate of the remaining kidney in each of the five cases, despite the fact that several types of kidney lesions were present in this series of patients.

Although the nephrectomy was followed by unquestioned reduction of the blood pressure in three of the five cases, there was slight or no improvement in the remaining two. Also, varying degrees of hypertension remained in all five patients.

Despite the fact that there was no ischemia of the remaining kidney following nephrectomy in three of the five patients, hypertension was found to be present in all of them. The presence of hypertension without renal ischemia in these patients, and the occasional demonstration of a similar state of affairs in the hypertensive patient without unilateral kidney disease (10), are suggestive evidence that renal ischemia may be a concomitant, but not necessarily a causative factor in the pathogenesis of human hypertension. The production of experimental renal hypertension in dogs (11) without the initial or later occurrence of renal ischemia further strengthens this view.

CONCLUSIONS

1. The total diodrast clearance was found to be reduced in four out of five patients having unilateral kidney disease with hypertension. It was more reduced in the affected kidney than in the normal one. The total inulin clearance was diminished in two, and normal in the remaining three patients.

2. The removal of the diseased kidney was followed by an increase in the renal blood flow and in the glomerular filtration rate of the remaining kidney in all five patients.

3. There was a significant blood pressure reduction in three of the five patients, but in none was there a complete return of the blood pressure to normal when last examined, despite the fact that in three of these patients there was no ischemia of the remaining kidney.

The authors wish to express their thanks to Doctors C. Johnson and W. J. Kerr of the University of California Medical School, and to Doctors L. Emge, T. Addis, W. Beckh and W. A. Sumner of the Stanford Medical School for their cooperation in making several of these patients available for study. We are indebted to Dr. A. Coxé of Stanford Medical School and Dr. G. Rusk of Mount Zion Hospital for their aid in the pathological study herein reported.

BIBLIOGRAPHY

1. Butler, A. M., Chronic pyelonephritis and arterial hypertension. *J. Clin. Invest.*, 1937, 16, 889.
2. Backer, N. W., and Walters, W., Hypertension associated with unilateral chronic atrophic pyelonephritis. Treatment by nephrectomy. *Proc. Staff Meet., Mayo Clin.*, 1938, 13, 118.

3. Braasch, W. F., Walters, W., and Hammer, H. J., Hypertension and the surgical kidney. *J. A. M. A.*, 1940, 115, 1837.
4. Leadbetter, W. F., and Burkland, C. E., Hypertension in unilateral renal disease. *J. Urol.*, 1938, 39, 611.
5. Crabtree, E. G., and Chaset, N., Vascular nephritis and hypertension. *J. A. M. A.*, 1940, 115, 1842.
6. Schroeder, H. A., and Fish, G. W., Studies on "essential" hypertension; the effect of nephrectomy upon hypertension associated with organic renal disease. *Am. J. M. Sc.*, 1940, 199, 601.
7. Wilson, C., and Byrom, F. B., Renal changes in malignant hypertension; experimental evidence. *Lancet*, 1939, 1, 136.
8. Friedman, B., Jarman, J., and Klemperer, P., Sustained hypertension following experimental unilateral renal injuries. Effects of nephrectomy. *Am. J. M. Sc.*, 1941, 202, 20.
9. Friedman, M., Selzer, A., and Rosenblum, H., The renal blood flow in coarctation of the aorta. *J. Clin. Invest.*, 1941, 20, 107.
10. Friedman, M., Selzer, A., and Rosenblum, H., The renal blood flow in hypertension. *J. A. M. A.*, 1941, 117, 92.
11. Friedman, M., Sugarman, H., and Selzer, A., The relationship of renal blood pressure and renal blood flow to the production of experimental hypertension. *Am. J. Physiol.*, 1941, 134, 493.

RADIOACTIVE IODINE AS AN INDICATOR IN THYROID PHYSIOLOGY. IV. THE METABOLISM OF IODINE IN GRAVES' DISEASE¹

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The use of radioactive iodine as an indicator in thyroid physiology has been described by Hertz, Roberts and Evans (1) and by Hertz, Roberts, Means and Evans (2, 3). Hamilton and Soley (4) have published data obtained by the use of radioactive iodine on goiter patients of several types.

We have administered radioactive iodine (mainly the 8-day isotope I^{131}), obtained by bombarding tellurium with deuterons in the Massachusetts Institute of Technology cyclotron, to 22 patients with Graves' disease and to 2 normal individuals. By this means we have investigated the absorption and excretion of labelled doses of iodine of various sizes, administered at various times during the course of pre-operative iodination and at various intervals before operation. In addition, the amount of iodine taken up by the thyroid and the distribution of the labelled iodine between the iodine-containing fractions of the thyroid have been determined in 19 cases.

PROCEDURE

Patients with Graves' disease were selected who had had no iodine previous to hospital admission. Labelled iodine was administered as sodium iodide by mouth and all other iodination was in the form of potassium iodide in saturated solution.

Glands were obtained at operation in 19 cases, at which time an estimate of the residual tissue was made. The glands were then fractionated according to the method of Harington (5) following alkaline hydrolysis. The acid-soluble and acid-insoluble fractions were then determined for both total and labelled iodine. It was found that the thyroid uptake of labelled iodine could not be directly determined from tissue samples because of the markedly non-uniform distribution of the labelled iodine. In about half the cases, relative measurements of the

amount of labelled iodine present in the thyroid were made by means of an external Geiger-Müller counter at various intervals after administration. The usual clinical observations were made, including the basal metabolic rate. Patients were at complete rest in the hospital and on an ordinary house diet. Fluid intake was not restricted.

The procedure for each uninodized patient was as follows: The basal metabolic rate was determined after a preliminary period of bed rest. Labelled iodine was then administered by mouth, and urines were collected in 12 cases. The uptake of labelled iodine by the thyroid was measured for 13 patients at various times after administration of the labelled iodine by means of an externally placed Geiger-Müller counter. No further iodine was given until these external measurements indicated that the thyroid iodine level had reached a relatively constant value. At this time routine iodination (minims 5 saturated solution of KI, twice daily) was begun. When maximal metabolic and clinical response had been reached 6 to 10 days later, 19 patients were subtotally thyroidectomized and an estimate of the residual thyroid tissue was made. A small portion of the excised gland was reserved for histologic study, and the remainder was fractionated chemically. The two fractions obtained, which we call "T" and "D" according to the notation of Salter (6), represent the thyroxine-like and diiodo-tyrosine-like fractions of the thyroid iodine. These fractions were assayed for both ordinary (total) iodine content and for labelled iodine.

The labelled iodine excreted in the urine was determined for most patients during the first few days following the administration of labelled iodine. Labelled blood iodine was determined in a few cases.

Cases 1, 2, 3, 4, 5, 9, and 10 received ordinary iodine prior to the administration of the labelled dose. The interval of preiodination given in Table I is the time between the first dose of ordinary iodine and the dose of labelled iodine. In only one of these cases, Case 10, was the patient totally iodinated by clinical standards before the administration of the labelled dose.

Cases 16 and 28 were normal persons and were, of course, not operated upon. In addition, Cases 15, 17, and 22 were not operated upon for clinical reasons.

The details of individual iodination procedure are given in Table I. The first clinical experiments were designed to afford data on the thyroid uptake from a labelled dose of iodine at various degrees of iodination

¹ This research was supported mainly by a grant from the John and Mary R. Markle Foundation, and also by a grant from the H. N. C. Gift for Medical and Surgical Research of Harvard University.

TABLE I
Metabolic and iodination data in Graves' disease and normal controls

Case number	Basal metabolic rate			Labelled dose	Days between labelled iodine administration and operation	Remarks
	Initial	Final	Interval of preiodinization			
			days	mgm.		
1	+45	+49	3	56	8	Partially iodinated Graves
2	+35	+17	3	6.4	5	Partially iodinated Graves
3	+50	+ 5	4	15	2	Partially iodinated Graves
4	+40	+25	15	60	10	Partially iodinated Graves
5	+30	+ 7	1	10	18	Partially iodinated Graves
6	+40	+16		21	8	Uniodinated Graves
7	+38	+25		60	5	Uniodinated Graves
8	+60	+43		325	7	Uniodinated Graves
9	+45	+20	2	4.8	3	Partially iodinated Graves
10	+48	+28	15	2.5	1	Completely iodinated Graves
11	+40	+18		2	8	Uniodinated Graves
12	+45	+31		2	8	Uniodinated Graves
13	+25	+15		0.23	10	Uniodinated Graves
14	+40	+24		16	11	Uniodinated Graves
15	+45	+45		0.23	Not operated	Uniodinated Graves
16	—	—		2.25	Not operated	Normal person
17	+37	+12		5	Not operated	Uniodinated Graves
18	+45	+25		0.1	11	Uniodinated Graves
22	+42	+26		0.55	Not operated	Uniodinated Graves
24	+45	+31		0.36	18	Uniodinated Graves
25	+33	0		0.36	13	Uniodinated Graves
26	+29	+ 5		0.35	19	Uniodinated Graves
27	+40	+22		0.35	13	Uniodinated Graves
28	—17			0.28	Not operated	Normal person

and at various dosage levels, and therefore present a cross-section of thyroid behavior.

RESULTS

Table II gives the results of the iodine fractionation of the thyroid tissue of the patients operated upon. It should be noted that the total iodine analysis cannot be compared directly with the labelled analysis, since the iodine in question has a different history.

Other results are shown in Figures 1 to 3.

A comparison of those patients who received ordinary iodine prior to the administration of labelled iodine with patients receiving similar doses of labelled iodine without any preiodinization reveals that the completely iodinated patient, Case 10, who received the labelled iodine the day before operation, had collected 2.0 per cent of the 2.5 mgm. labelled dose, in contrast to the almost complete collection at that time by a previously uniodinated patient, *e.g.*, Cases 11 or 12. With less complete iodination and a comparable labelled dose, as in Case 2, the value is intermediate, and in Case 9, where only one dose of 300 mgm. of

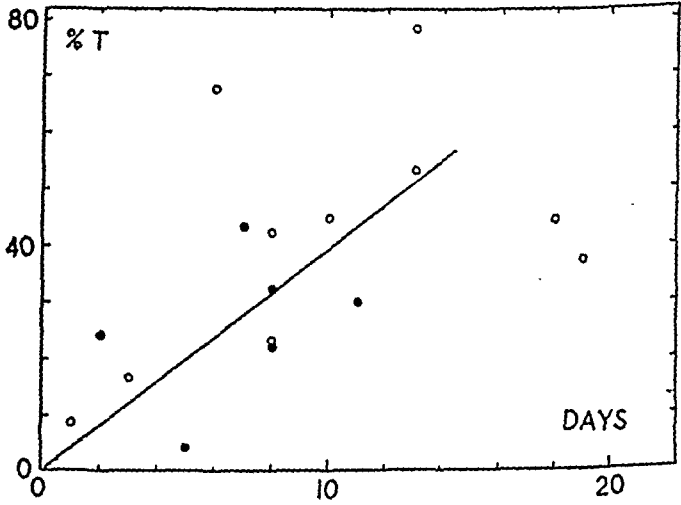


FIG. 1. LABELLED IODINE IN THE T FRACTION OF THYROID IODINE VS. TIME ELAPSING BETWEEN ADMINISTRATION OF LABELLED IODINE AND OPERATION

The open circles are for labelled iodine doses of less than 5 mgm. and the full circles for doses greater than 5 mgm. In general, this curve shows an increasing proportion in the T fraction as the time increases, in agreement with the work of Gutman, Benedict, Baxter, and Palmer (7).

KI had been given, no diminution in the collection of the subsequent labelled dose is evident.

TABLE II
Thyroid iodine analysis

Case number	Thyroid weight	Total iodine analysis			Labelled iodine analysis			Labelled iodine balance		
		Total iodine	D	T	Total iodine	D	T	Thyroid	Urine	Sum
	grams	mgm.	per cent	per cent	mgm.	per cent	per cent	per cent		
1	74	65.6	65	35	0.41	68	32	0.7	>19	>20
2	50	11.7	89	11	0.59	96	4	9.2	>64	>73
3	46	6.2	81	19	1.19	77	25	8.0	87	96
4	31	5.2	59	41	<1.37			<2.3	>50	>50
5	39	7.8	76	24	<.03			<0.3		
6	46	14.0	72	28	1.25	78	22	5.9	67	73
7	26	10.5	65	35	<1.9			<2.8	>96	>96
8	34	7.1	85	15	0.15	57	43	0.05	>95	>95
9	38	11.8	71	29	2.46	84	16	55		
10	47	11.2	72	28	0.049	92	8	2.0		
11	34	7.7	66	34	0.62	58	42	31		
12	117	6.1	78	22	0.55	77	23	28		
13	34	2.9	78	22	0.031	56	44	14		
14	25	17.5	64	36	0.034	70	30	0.22		
18	27	12.6	79	21	0.025	32	68	26	10	72*
24	58	31.5	73	27	0.069	57	43	19	34	63*
25	24	6.9	68	32	0.075	22	78	21	37	67*
26	40	19.0	80	20	0.107	63	37	29	31	69*
27	33	18.6	66	34	0.090	47	53	24	28	59*

* These values represent the sum of urinary iodine and initial thyroid uptake, the latter being obtained from the value found at operation and the shape of the curve for external GM counter measurements of the thyroid iodine.

In those cases which were given larger amounts of labelled iodine the thyroid collection was lower than with small doses, and was correspondingly decreased by preiodinization.

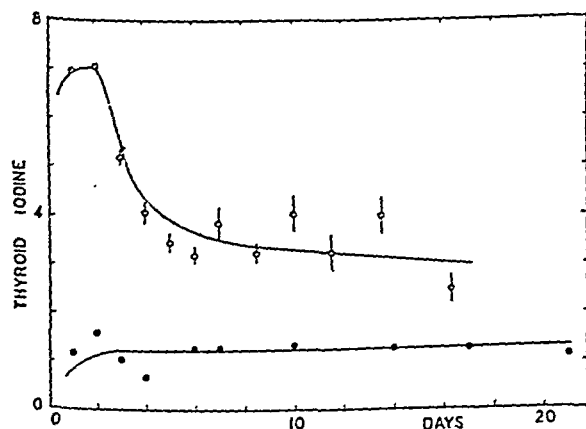


FIG. 2. COMPOSITE CURVE FOR THYROID IODINE UPTAKE IN 12 UNTREATED PATIENTS WITH CLASSIC GRAVES' DISEASE

Iodine is taken up rapidly by the toxic thyroid and then gradually released. The lower curve is for 2 normal persons. The scale of ordinates is arbitrary, but both curves are to the same scale. One completely iodinated patient with Graves' disease showed an uptake similar to that of normal persons.

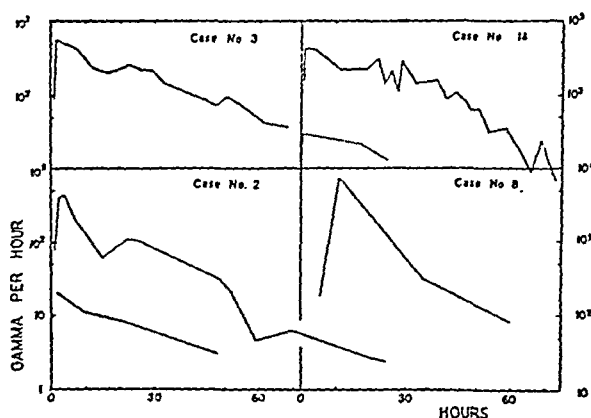


FIG. 3. URINARY EXCRETION OF LABELLED IODINE IN TYPICAL CASES

The lower curves for Cases 2 and 14 show the labelled iodine level in the blood in gamma per cent.

DISCUSSION

The main features of these results are in agreement with previous data obtained both with animals and with patients. The rapidity of the collection of iodine in the thyroid after oral administration is such as to bear out the hypothesis that the time for initial thyroid iodine collection is simply the time taken by the iodine to reach the thyroid. The large percentage uptake from a

small dose is even more striking in Graves' disease than in our earlier experiments on rabbits whose thyroids were made hyperplastic with anterior pituitary thyrotropic hormone (2, 3). The approximate balance with small doses of administered iodine on the one hand, and initial thyroid iodine plus urinary iodine on the other hand, shows that there is under these conditions no other principal iodine-storing organ. This is in agreement with previously reported analyses of rabbit organs in which the 25-minute isotope was used. With larger doses this balance is incomplete and iodine finds its way to other organs.

The relations between dosage and initial collection appear to be somewhat as follows: The untreated Graves' disease thyroid will collect up to an initial maximum of 80 to 90 per cent of a "small" dose (2 mgm. or less). For slightly larger doses (14 mgm.), this percentage may fall somewhat (4). If the final amounts found in our own patients at the 14 mgm. dosage level can be extrapolated back to give the initial collection, according to the curve for the small dosages in Figure 2, the conclusion can be drawn that in most patients the initial thyroid iodine collection does not exceed about 5 mgm. from any one dose. This is in agreement with the finding in rabbits that a dosage ceiling can be found above which the absolute collection remains constant (2). Thus, very large doses give only a small percentage uptake.

The difference in shape between the curves for thyroid iodine uptake in untreated cases and normal persons shows that whereas, in the latter, little if any loss of iodine occurs within the next few days following the administration of iodine; in the untreated cases, which take up large amounts of iodine, there is a definite loss of iodine from the gland during this period. This is of considerable interest, since in these patients we are effectively studying the fate of an amount of iodine similar to what may be encountered in the diet. Thus we see that the thyroid in Graves' disease does not tend to become saturated with iodine by accumulating small amounts, but tends rather to take up this iodine and later to secrete it, perhaps in calorigenic form.

To summarize our picture of the metabolism of a single dose of iodine in Graves' disease, we may take as an example Case 18. This patient

was an unmarried woman of 25 showing a basal metabolic rate of $+45$, with classic symptoms and physical findings of moderate Graves' disease. Following 4 days' rest in bed, without specific medication, 95 micrograms of labelled iodine were administered orally. The basal metabolic rate remained unchanged by this amount of iodine, and observations of the labelled iodine uptake in the thyroid were made for about 2 weeks. Urine was collected in 12-hour specimens for several days after the administration of the labelled iodine. No other iodine was administered during the period of observation. The results of the external measurements gave a curve by means of which the initial collection was found to be 80 per cent of the administered dose after the curve was normalized by measurements of the excised thyroid. The urinary excretion accounted for 11 per cent. Blood samples were taken on the 3rd and 10th days. Iodine was administered routinely from the 9th day on. The basal metabolic rate showed a response from $+45$ to $+25$. The patient was operated on the 15th day, and the gland was found to be diffusely hyperplastic, weighing 27 grams. The thyroid was analyzed and the results of the analysis are given in Table II. The total labelled iodine was 25.1 micrograms, of which 62.5 per cent was in the T fraction—an unusually high percentage. The total iodine was 12.6 mgm., of which only 21 per cent was in the T fraction. This conforms to the concept that older iodine is more likely to be in the T fraction than new iodine.

The failure of the 55 per cent of the iodine, which was initially taken up by the thyroid and later released, to appear in the urine means that it is either elsewhere in the body or has been excreted by another route. However, the latter possibility is remote, as indicated by the low blood level, and by Hamilton and Soley's failure ever to observe more than a few per cent in the feces (4). Thus it appears that this iodine is distributed through tissues other than the thyroid and the blood. Attempts to locate this iodine by means of the external counter met with no success. It therefore seems likely to us that it is fairly uniformly and diffusely distributed since, if this were the case, the activity would be too weak to be detected. The problem now arises as to whether this iodine is in inorganic or organic combination,

and especially as to whether it is in calorogenic form. Results of the analysis of the labelled blood iodine on the 10th day indicate that the major portion of the labelled blood iodine is in protein combination.

SUMMARY

Radioactive iodine was used as a tracer in the study of the metabolism of iodine in Graves' disease. In a series of 22 thyrotoxic patients and 2 normal persons, we have studied the urinary excretion, the thyroid uptake and retention, and the chemical distribution of the thyroid iodine. In agreement with results previously obtained with animals, we find the largest percentage uptakes in the thyroid at low dosage levels. Preiodinization with commonly used clinical dosage causes a decrease in the subsequent thyroid collection of a labelled dose. The hyperplastic thyroid of Graves' disease may collect initially 80 per cent or more from a sufficiently small dose (2 mgm.). Urinary excretion accounts for most of the remaining iodine. Analysis of the thyroid after operation into thyroxine-like and non-thyroxine-like fractions shows a general trend of the labelled iodine to be increasingly in the former fraction as the time following administration increases. Urinary and blood levels of labelled iodine are given.

We wish to express our gratitude to Professor Robley D. Evans and Dr. James H. Means for their continued interest and encouragement.

BIBLIOGRAPHY

1. Hertz, S., Roberts, A., and Evans, R. D., Radioactive iodine as an indicator in thyroid physiology. *Proc. Soc. Exp. Biol. and Med.*, 1938, 38, 510.
2. Hertz, S., Roberts, A., Means, J. H., and Evans, R. D., Radioactive iodine as an indicator in thyroid physiology. II. Iodine collection by normal and hyperplastic thyroids in rabbits. *Am. J. Physiol.*, 1940, 128, 565.
3. Hertz, S., and Roberts, A., Radioactive iodine as an indicator in thyroid physiology. III. Iodine collection as a criterion of thyroid function in rabbits injected with thyrotropic hormone. *Endocrinology*, 1941, 29, 82.
4. Hamilton, J. G., and Soley, M. H., Studies in iodine metabolism by the use of a new radioactive isotope of iodine. *Am. J. Physiol.*, 1939, 127, 557.
5. Harington, C. R., and Randall, S. S., Observations on the iodine-containing compounds of the thyroid gland. *Biochem. J.*, 1929, 23, 373.
6. Salter, W. T., *The Endocrine Function of Iodine*. Harvard University Press, Cambridge, 1940.
7. Gutman, A. B., Benedict, E. M., Baxter, B., and Palmer, W. W., The effect of administration of iodine on the total iodine, inorganic iodine, and thyroxine content of the pathological thyroid gland. *J. Biol. Chem.*, 1932, 97, 303.
8. Hertz, S., Means, J. H., and Williams, R. H., Graves' disease with dissociation of thyrotoxicosis and ophthalmopathy. *West. J. Surg.* (In press.)

RADIOACTIVE IODINE AS AN INDICATOR IN THYROID PHYSIOLOGY.

V. THE USE OF RADIOACTIVE IODINE IN THE DIFFERENTIAL DIAGNOSIS OF TWO TYPES OF GRAVES' DISEASE^{1, 2}

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(Received for publication July 28, 1941)

Recently, attention has been called to the importance of a special variety of Graves' disease which differs from the classic variety in that the ophthalmic manifestations become dissociated from the thyrotoxic (1). The characteristics of this special type, to which we shall refer as the ophthalmopathic type of Graves' disease, are: predominantly ocular symptomatology such as diplopia, chemosis, and edema; mild or absent thyrotoxic manifestations; normal or only slightly elevated basal metabolic rate; rapid decrease in metabolic rate to substandard levels upon administration of iodine; absence or relatively slight degree of enlargement of thyroid; and positive thyrotropic assays of blood and urine. The behavior of cases of classic Graves' disease toward radioactive iodine has been studied previously (2). We will now present parallel studies on the special ophthalmopathic type.

PROCEDURE

On the basis of previous experience (2), small doses of labelled iodine (less than 2 mgm.) were orally administered before any other treatment was given, especially before any other iodine. The thyroid iodine collection of the patient was followed by means of an externally placed Geiger-Müller counter and, in addition, urines were collected for 72 hours following the administration of the labelled iodine. (No appreciable iodine is excreted after this interval.)

The ophthalmopathic patients were compared directly with the classic type Graves' disease patients both as to thyroid collection and urinary excretion. Normal controls were also followed. The classic type patients were operated upon and the thyroid labelled iodine determined directly on the excised gland. The ophthalmopathic group was not operated upon, since operation is thought to be contraindicated in this group.

¹ This research was supported principally by a grant from the John and Mary R. Markle Foundation, and also by a grant from the H. N. C. Gift for Medical and Surgical Research of Harvard University.

² Presented at the American Society for Clinical Investigation meeting in Atlantic City, New Jersey, May 5, 1941.

RESULTS

The results of the external gamma ray measurements on the thyroid are shown in Figure 1. In this figure are plotted composite thyroid collection curves for each type of patient, as well as for normal patients. The results are given for 6 classic and 5 ophthalmopathic type patients and for 2 normal persons. The absolute value of the thy-

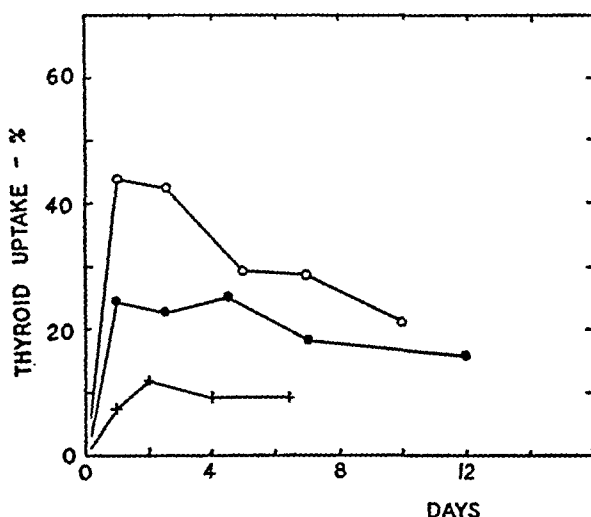


FIG. 1. THYROID UPTAKE AS A FUNCTION OF TIME AFTER ADMINISTRATION OF THE LABELLED DOSE

The open circles are composite points for the classic type, the full circles for the ophthalmopathic type and the crosses for normal persons.

roid collection of labelled iodine in the ophthalmopathic group is not accurate and may be in error by as much as 50 per cent in any one case, although the composite has a smaller probable error. This error is due to individual variation in thyroid size and anatomy. The relative differentiation between the two types is borne out by the iodine excretion in the urine as shown in Figure 2.

The initial basal metabolic rates and the final levels after complete iodination (after the meas-

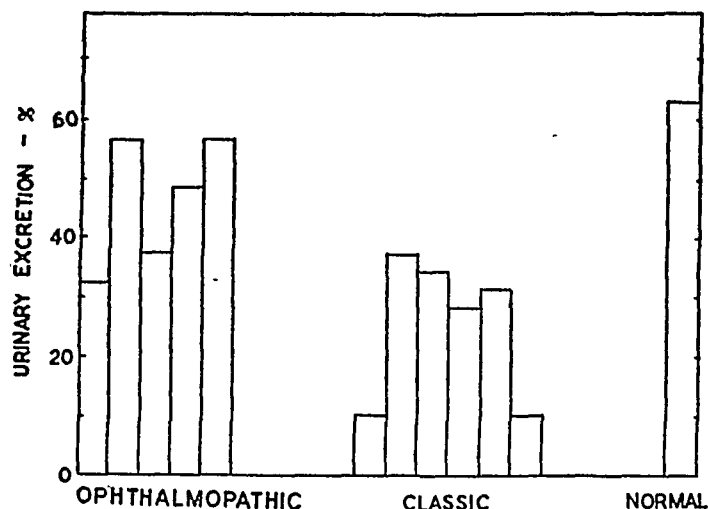


FIG. 2. PERCENTAGE OF THE SMALL LABELLED DOSE OF IODINE EXCRETED WITHIN 72 HOURS BY THE PATIENTS WITH CLASSIC AND OPTHALMOPATHIC TYPES OF GRAVES' DISEASE AND ONE NORMAL PERSON

urements on the labelled dose were completed) are given in Table I.

TABLE I

Case	Type	Initial basal metabolic rate	Final basal metabolic rate	Iodine excretion (per cent)
21	Ophthalmopathic	- 1	- 2	32
23	Ophthalmopathic	+46	- 8	56
20	Ophthalmopathic	+23	-14	37
19	Ophthalmopathic	+15	- 9	48
29	Ophthalmopathic	+ 2	- 9	56
22	Classic	+42	+26	10
25	Classic	+33	0	37
24	Classic	+55	+31	34
27	Classic	+40	+22	28
26	Classic	+29	+ 5	31
18	Classic	+46	+25	10

DISCUSSION

The difference in behavior between the two types of patients with Graves' disease is seen to be as follows: The ophthalmopathic group takes up less of the test dose of iodine in the thyroid and excretes more in the urine than the classic group, its behavior being in fact intermediate between the classic group and the normal. From this alone it might appear that the so-called ophthalmopathic group is merely a group of less severe classic cases.

That this is not the case is indicated by a study of the metabolic rates and the degree of response to complete iodination. One of the distinguishing characteristics of the ophthalmopathic group is its exceptionally large response to iodination, frequently to minus levels. A correlation study of the basal metabolic rate after iodination shows that the rank correlation coefficient between the final basal metabolic level and the urinary excretion is -0.66 for all cases except the normal cases. This indicates that high urinary excretion of the test dose is well correlated with the level of basal metabolic response. The probability that this correlation is due to statistical fluctuation is 0.02.

Since the urinary excretion of a test dose is thus a good index of the type to which the patient belongs, this offers an additional convenient differential diagnostic aid. The use of labelled iodine for this purpose is not essential, provided that chemical methods for assaying the urinary iodine are available. Labelled iodine is merely a convenience for this purpose because of the great sensitivity and ease of the method.

SUMMARY

The ophthalmopathic type of Graves' disease patient excretes more iodine from a 2 mgm. (or less) test dose and takes up less in the thyroid than the classic Graves' disease patient. The urinary iodine excretion can be used as a diagnostic aid in distinguishing the two types of patients.

We are grateful for the generous interest and encouragement of Professor Robley D. Evans and Dr. J. H. Means.

BIBLIOGRAPHY

1. Hertz, S., Means, J. H., and Williams, R. H., Graves' disease with dissociation of thyrotoxicosis and ophthalmopathy. *Western J. Surg.*, 1941, 49, —.
2. Hertz, S., Roberts, A., and Salter, W. T., Radioactive iodine as an indicator in thyroid physiology. IV. The metabolism of iodine in Graves' disease. *J. Clin. Invest.*, 1942, 21, 25.

THE RÔLE OF THE ADRENAL CORTEX IN ACUTE ANOXIA^{1,2}

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We were led to investigate this phase of adrenal cortical function by Evans' observation (1, 2) that liver glycogen and blood glucose increased strikingly in rats exposed to low atmospheric pressure. Evans' studies clearly indicated that the phenomenon did not occur in the absence of the adrenal cortex. However, treatment with adrenal cortical hormone preparations which were then available did not restore to normal the response of adrenalectomized animals. Recently Armstrong and Heim (3) reported that hypertrophy of the adrenals occurred in rabbits which were exposed repeatedly to low atmospheric pressure. Furthermore, it has been suggested that the asthenia and hypotension which have been noted in aviators who have served continuously as pilots bear a resemblance to the clinical syndrome associated with adrenal cortical insufficiency (4).

In this investigation rats, rabbits, dogs, monkeys and human subjects have been exposed for single periods of 1.5 to 24 hours to mixtures of oxygen and nitrogen with the partial pressure of oxygen adjusted to correspond to altitudes of 11,000 to 34,000 feet (Table I). Exposure of

larger animals and human subjects was accomplished at low cost by using an apparatus which permitted the mixing of nitrogen with air (5).

METHODS

Male albino rats, 150 to 200 grams in weight, of the Sprague Dawley strain, were maintained on a diet of Purina Dog Chow. Bilaterally adrenalectomized rats were treated in addition with 1 per cent sodium chloride added to the drinking water, or with one 125 mgm. pellet of desoxycorticosterone acetate⁶ implanted subcutaneously, or with 1 cc. of adrenal cortical extract⁷ injected daily. Rats were exposed to mixtures of oxygen and nitrogen in metabolism cages enclosed in glass bell-jars.⁸ Samples of blood were obtained from the cut edge of the liver⁹ at the time that specimens of liver and muscle were removed for glycogen determination. The Folin-Malmros micro method (6) was used for the blood sugar determinations. Liver and muscle glycogen were determined by the method of Good, Kramer and Somogyi (7) after the tissue had been frozen in liquid nitrogen.

Male albino rabbits, weighing approximately 2 kgm., were maintained on a diet of B-B Complete Rabbit Food Pellets (Maritime Milling Co., Inc.). Blood samples were obtained under oil from the heart. Rabbits as well as dogs and monkeys were exposed to mixtures of oxygen and nitrogen in metabolism cages enclosed in well ventilated glass chambers.⁸

Male dogs, weighing 10 to 15 kgm. were kept in metabolism cages and fed a constant diet consisting of 350 grams of raw, ground beef to which 50 cc. of Pet Milk was added. The technique used in the care of the dogs and in the collection of specimens has been described (8).

TABLE I
Altitude equivalent of oxygen-nitrogen mixtures
used in these experiments

Oxygen concentration	Partial pressure of oxygen	Altitude above sea level
per cent of dry gas	mm. of Hg	feet
20.9	159	0
13.8	105	11,000
12.8	98	13,000
10.5	80	18,000
7.1	54	27,000
5.2	39	34,000

¹ An abstract of this paper was presented before the 56th Annual Meeting of the Association of American Physicians, Atlantic City, May 6, 1941.

² This study was aided by a grant from the Committee on Research in Endocrinology, National Research Council.

³ John D. Archbold Fellow-in-Medicine.

⁴ John H. Harris Fellow-in-Medicine.

⁵ Dazian Foundation Fellow-in-Residence.

⁶ The pellets of crystalline desoxycorticosterone acetate and the desoxycorticosterone acetate in sesame oil used in this study were provided through the courtesy of Dr. E. Oppenheimer of the Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

⁷ The adrenal cortical extract used in this study was generously supplied by Dr. David Klein of the Wilson Co., Chicago, Illinois.

⁸ The mixtures of oxygen and nitrogen were circulated through the cages at a rate which was rapid enough to prevent the accumulation of over 0.5 per cent carbon dioxide.

⁹ The level of glucose in the blood from the cut edge of the liver was found to be the same as that in the blood obtained from the heart.

Bilaterally adrenalectomized dogs were maintained in good condition with 3 to 4 subcutaneously implanted pellets of desoxycorticosterone acetate (9). The methods used in the analysis of blood and urine have been described (10).

Monkeys (*Macacus rhesus*), weighing approximately 5 kgm., were maintained on a diet of milk, bananas, bread and raw vegetables. Samples of blood were obtained under oil from the heart.

Normal human subjects, 25 to 40 years of age, were maintained on a constant diet in which the proportions of carbohydrate, fat and protein were 5.0:1.5:1.25. The caloric content of the diet was adjusted to meet the metabolic requirements of each individual, while the mineral content was kept constant for each subject. Following a period of 5 days on the diet and an overnight fast of 15 hours, the volunteers were exposed in an oxygen tent for 5 hours. The gas mixing apparatus was modified for human use by introducing larger meters¹⁰ and by supplying room air to the mixer under slightly positive pressure with a small air pump. The air mixture was circulated and cooled through a conventional oxygen tent. The rate of ventilation (20 to 25 liters per minute) was sufficiently rapid to keep the carbon dioxide content of the air in the tent below 1 per cent. The subjects were kept in bed before, during and for a few hours after the exposure. Samples of tent air were collected at the same time as the samples of expired air. In some instances intravenous glucose tolerance tests¹¹ were performed during anoxia. Blood samples for chemical analyses were removed under oil from the brachial artery. The hydrogen ion concentration of arterial blood was measured with an improved glass electrode designed for use with the Beckman pH meter.

OBSERVATIONS

Carbohydrate metabolism

1. Effect of exposure for 24 hours to oxygen at a partial pressure of 80 mm. Hg

The increase in blood sugar and liver glycogen in rats exposed to a mixture of oxygen and nitrogen of low oxygen tension (Table II) was similar to that reported by Evans in rats exposed to low barometric pressure and similar oxygen tension (1). A significant increase in the excretion of

¹⁰ We are indebted to Mr. M. Stockton of the American Meter Co., Albany, New York, for the meters (AL-19-1) which were used in this study.

¹¹ In this test 0.5 gram of glucose per kgm. of body weight was injected intravenously as a 20 per cent solution in distilled water. The flow was adjusted so that the infusion was completed in 30 minutes. Capillary blood for sugar determinations was taken in the fasting state and at 30-minute intervals during a 4-hour period following the glucose infusion.

TABLE II

Effect of anoxia on the carbohydrate metabolism of normal rats: exposure for 24 hours to oxygen at a partial pressure of 80 mm. Hg

	Control	Anoxia
Number of animals	4	4
Blood sugar	70*	104
mgm. per 100 cc.	(53-78)†	(87-123)
Liver glycogen	40	1,260
mgm. per 100 grams	(20-50)	(1,010-1,630)
Muscle glycogen	440	560
mgm. per 100 grams	(270-620)	(520-620)
Urine nitrogen	70	79
mgm. per 100 grams body weight per 24 hours	(pooled)	(pooled)

* The first figure indicates the average for the group.

† The figures in parentheses indicate the range for the group.

nitrogen was demonstrated only in experiments at lower oxygen tensions (discussed below). The glycogen which had accumulated in the liver of rats exposed to low oxygen tension disappeared after a subsequent 12-hour fast at approximately the same rate at which glycogen disappears from the livers of normal rats during such a fast.

The blood sugar of dogs exposed to low oxygen tension for 24 hours did not increase. Changes in the liver glycogen of exposed dogs were not considered significant because of the large spread in the values for fasting liver glycogen of control dogs. There was, however, a definite increase in the renal excretion of nitrogen and phosphorus of dogs during exposure to low oxygen tension for 24 hours, suggesting that protein catabolism had increased somewhat. It is probable that the high liver glycogen of dogs fasted for 24 hours compared to the low liver glycogen of other species (Figure 1) is related to the marked difference in the diet of these animals (Table III), since dogs were maintained on a diet containing a high proportion of protein, whereas other experimental

TABLE III

Approximate composition of the diets which were used in preparation for exposure to low oxygen tension

Species	Per cent of dry weight		
	Carbohydrate	Protein	Fat
Rat.....	68	25	7
Rabbit.....	75	22	3
Dog.....	0	73	27
Monkey.....	80	10	10
Human.....	65	16	19

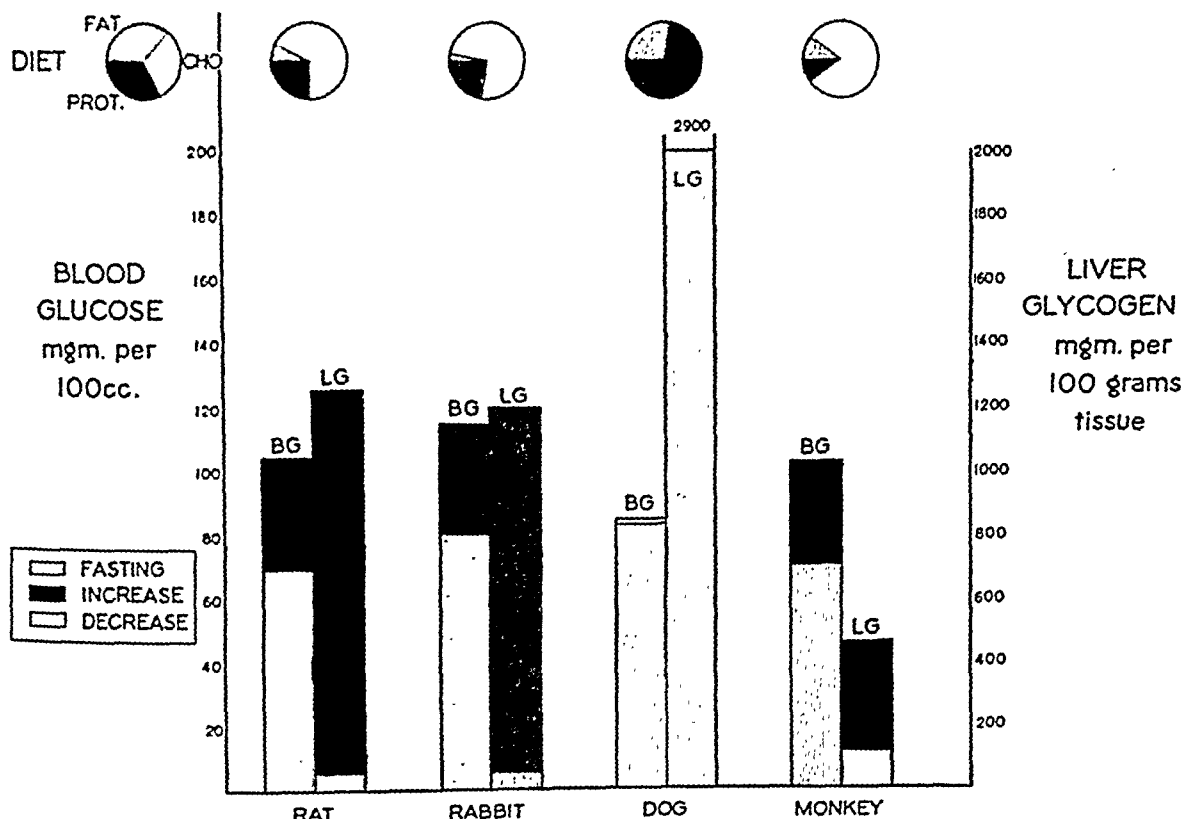


FIG. 1. THE EFFECT OF ANOXIA (24 HOURS AT 10.5 PER CENT O_2) ON LIVER GLYCOGEN AND BLOOD GLUCOSE OF VARIOUS SPECIES

The blood glucose and liver glycogen were determined in at least six animals of each species charted. The average values of animals exposed to an oxygen concentration of 10.5 per cent were charted and the average values of control animals were superimposed. The increase in blood glucose and liver glycogen is shown in solid black.

animals and human subjects were maintained on a diet rich in carbohydrate.

The blood sugar and liver glycogen of rabbits and monkeys exposed to a low oxygen tension for 24 hours were considerably greater than the blood sugar and liver glycogen of control animals that had fasted for 24 hours. Nitrogen excretion was not studied in these animals. It was not feasible to expose human subjects for 24 hours.

2. Relation of the degree of anoxia to the change in carbohydrate metabolism

When rats were exposed to various oxygen tensions for 24 hours (Figure 2) the glycogen in the liver varied inversely as the oxygen tension. Thus in 14 per cent oxygen (105 mm. Hg partial pressure) the increase in liver glycogen was slight but

definite. At the other extreme of 7 per cent oxygen (54 mm. Hg partial pressure) not only liver glycogen but also blood sugar increased greatly.

3. Relation of acapnia to changes in carbohydrate metabolism during anoxia

In one series of experiments 5 per cent carbon dioxide (40 mm. Hg partial pressure) was added to the mixture of oxygen and nitrogen. In the presence of carbon dioxide during anoxia liver glycogen increased definitely but less than it did without the addition of carbon dioxide (Table IV). The fact that the addition of carbon dioxide modified the increase in carbohydrate storage is compatible with the observation that anoxemia is decreased by the administration of carbon dioxide (11).

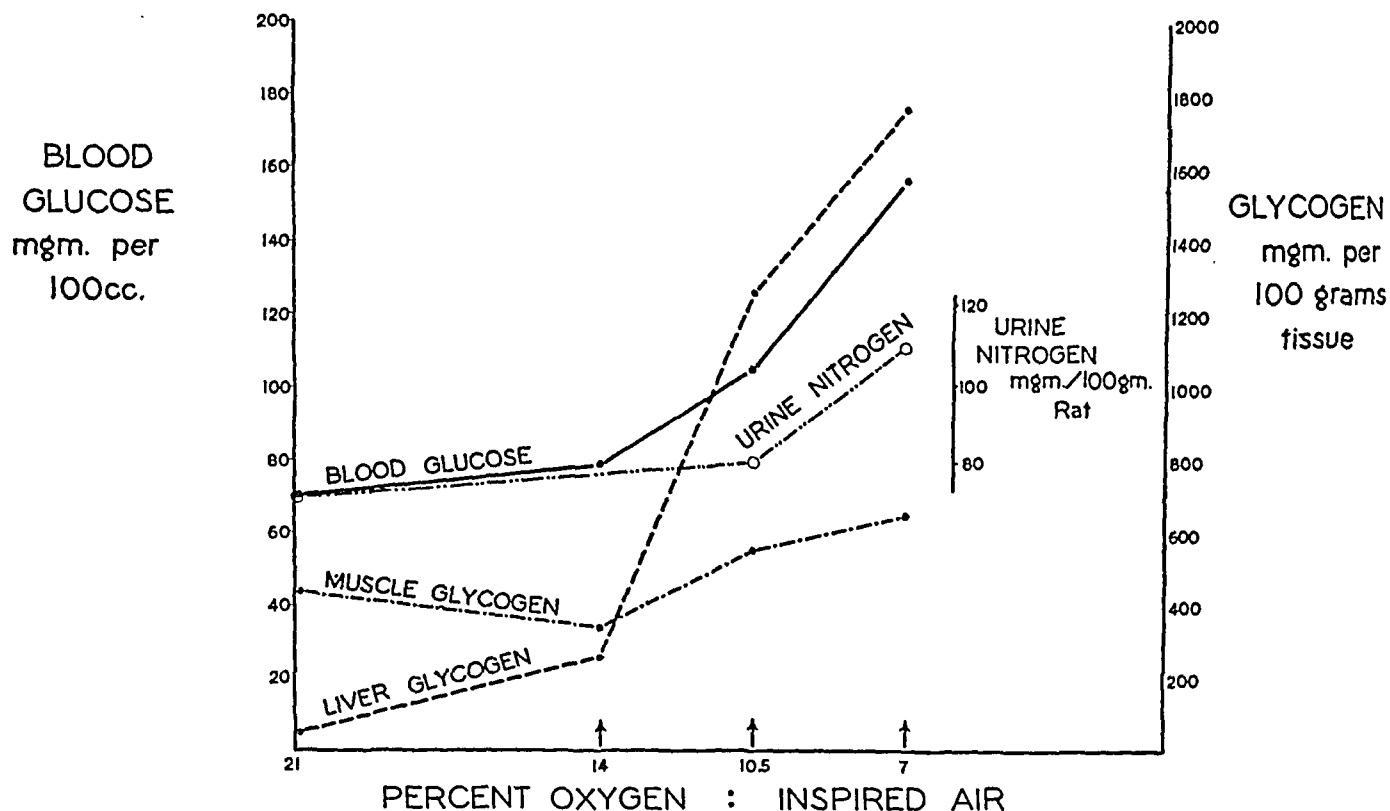


FIG. 2. The EFFECT OF ANOXIA (24 HOURS) ON BLOOD GLUCOSE, LIVER AND MUSCLE GLYCOGEN OF NORMAL RATS

TABLE IV

Changes in carbohydrate metabolism during anoxia: the effect of adding carbon dioxide to the mixture of oxygen and nitrogen

Number of rats used	Carbon-dioxide partial pressure	Oxygen partial pressure	Blood sugar	Liver glycogen	Muscle glycogen
	mm. Hg	mm. Hg	mgm. per 100 cc.	mgm. per 100 grams	mgm. per 100 grams
4	0	159	70	60	440
4	0	80	104	1,260	560
4	40	80	91	500	480

TABLE V

Effect of anoxia on the carbohydrate metabolism of adrenalectomized rats: exposure for 24 hours to oxygen at a partial pressure of 80 mm. Hg*

	Control (adrenalectomized)	Anoxia (adrenalectomized)
Number of animals	3	3
Blood sugar	54†	42
mgm. per 100 cc.	(45-66)**	(36-50)
Liver glycogen	30	20
mgm. per 100 grams	(20-40)	(20-30)
Muscle glycogen	250	90
mgm. per 100 grams	(120-380)	(70-100)
Urine nitrogen	74	55
mgm. per 100 grams body weight per 24 hours	(pooled)	(pooled)

* Adrenalectomized rats were maintained with 1 per cent sodium chloride added to the drinking water.

† The first figure indicates the average for the group.

** The figures in parentheses indicate the range for the group.

4. Relation of the adrenal cortex to carbohydrate metabolism during anoxia

In adrenalectomized rats exposed to low oxygen tension (80 mm. Hg partial pressure) for 24 hours liver glycogen did not increase (Table V). This confirms the studies of Evans (1, 2). Muscle glycogen and blood glucose of exposed adrenalectomized rats were below the low fasting level of unexposed adrenalectomized animals. A number of adrenalectomized rats maintained on sodium chloride did not survive the exposure.

5. Effect of adrenal cortical hormone therapy on the carbohydrate metabolism of adrenalectomized rats during anoxia

Injections of aqueous adrenal cortical extract at hourly intervals or a suspension of this material in oil at 6-hour intervals were followed by a rise in the blood sugar and liver glycogen of adrenalectomized rats during anoxia (Figures 3, 4). With this treatment adrenalectomized rats were enabled to tolerate a 24-hour exposure to an oxygen partial pressure of 80 mm. Hg without signs of hypoglycemia. Treatment with adequate quantities of potent adrenal cortical extract thus restored the ability of adrenalectomized animals to respond to anoxia in a manner comparable to con-

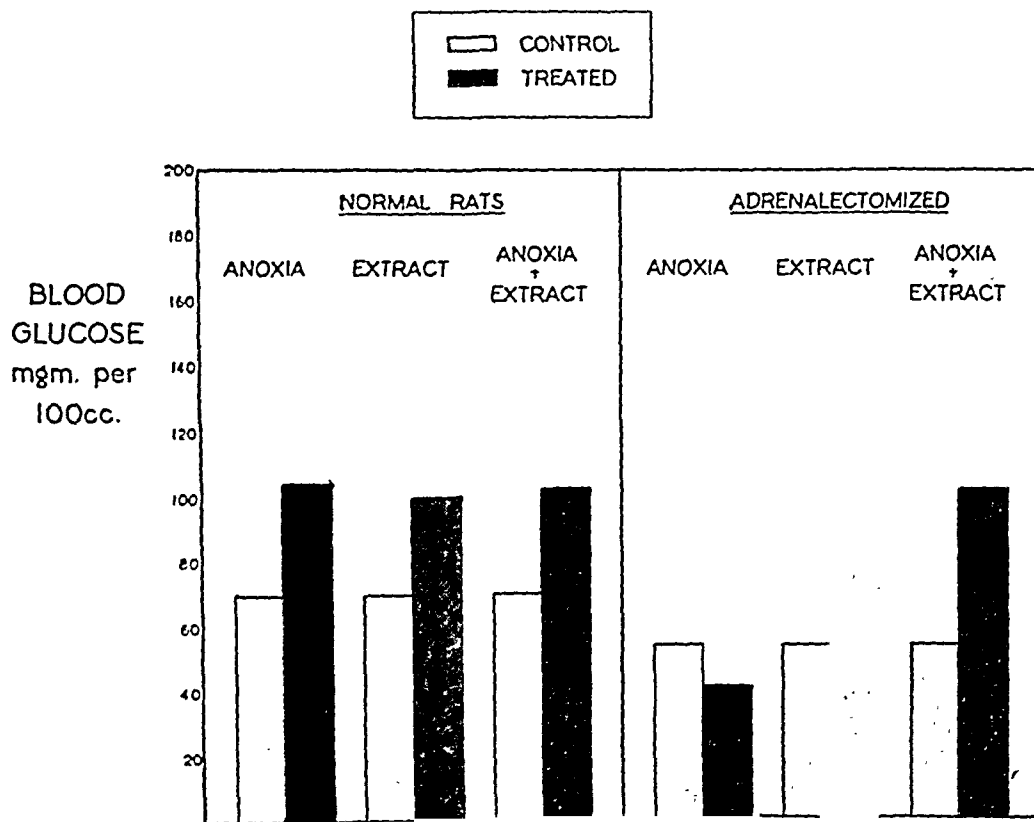


FIG. 3. EFFECT OF ANOXIA (24 HOURS AT 10.5 PER CENT O₂) AND ADRENAL CORTICAL EXTRACT TREATMENT ON BLOOD GLUCOSE

trols. There was no increase in the blood sugar or liver glycogen of adrenalectomized rats treated with sodium chloride or desoxycorticosterone acetate and exposed to low oxygen tension.

6. Effect of adrenal cortical hormone therapy on the carbohydrate metabolism of normal unexposed rats

It has been shown by Long *et al.* (12) that the administration of certain adrenal cortical hormones tends to augment carbohydrate storage and nitrogen excretion. The liver glycogen content is of the same order of magnitude in normal and adrenalectomized rats treated with adrenal cortical extract or 11-dehydro-17-hydroxycorticosterone¹² as it is in normal rats exposed to low oxygen tensions (Table VI). The increase in nitrogen excretion of normal and adrenalectomized animals

¹² We are indebted to Dr. E. C. Kendall of the Mayo Clinic, Rochester, Minnesota, for the crystalline 11-dehydro-17-hydroxycorticosterone (Compound E).

TABLE VI

The effect of adrenal cortical hormone therapy on carbohydrate metabolism of rats

Treatment	Control	Anoxia, 24 hours at 80 mm. Hg oxygen partial pressure	Adrenal cortical extract, 10 cc. in 1 cc. oil	11-dehydro-17-hydroxycorticosterone, 6 mgm. in 1 cc. oil	Desoxycorticosterone acetate, 6 mgm. in 1 cc. oil
Number of animals	3	3	3	1	3
Blood sugar mgm. per 100 cc.	70	104	103	105	63
Liver glycogen mgm. per 100 cc.	60	1,260	1,870	1,240	20
Muscle glycogen mgm. per 100 cc.	440	560	300	270	140
Urine nitrogen mgm. per 100 grams body weight per 24 hours	70	79	78	103	68

treated with 11-dehydro-17-hydroxycorticosterone exceeded the increase observed during anoxia.

7. Effect of exposure for 1.5 to 6 hours to reduced oxygen tension

Preliminary observations suggested that in some animals a hypoglycemic phase might precede the

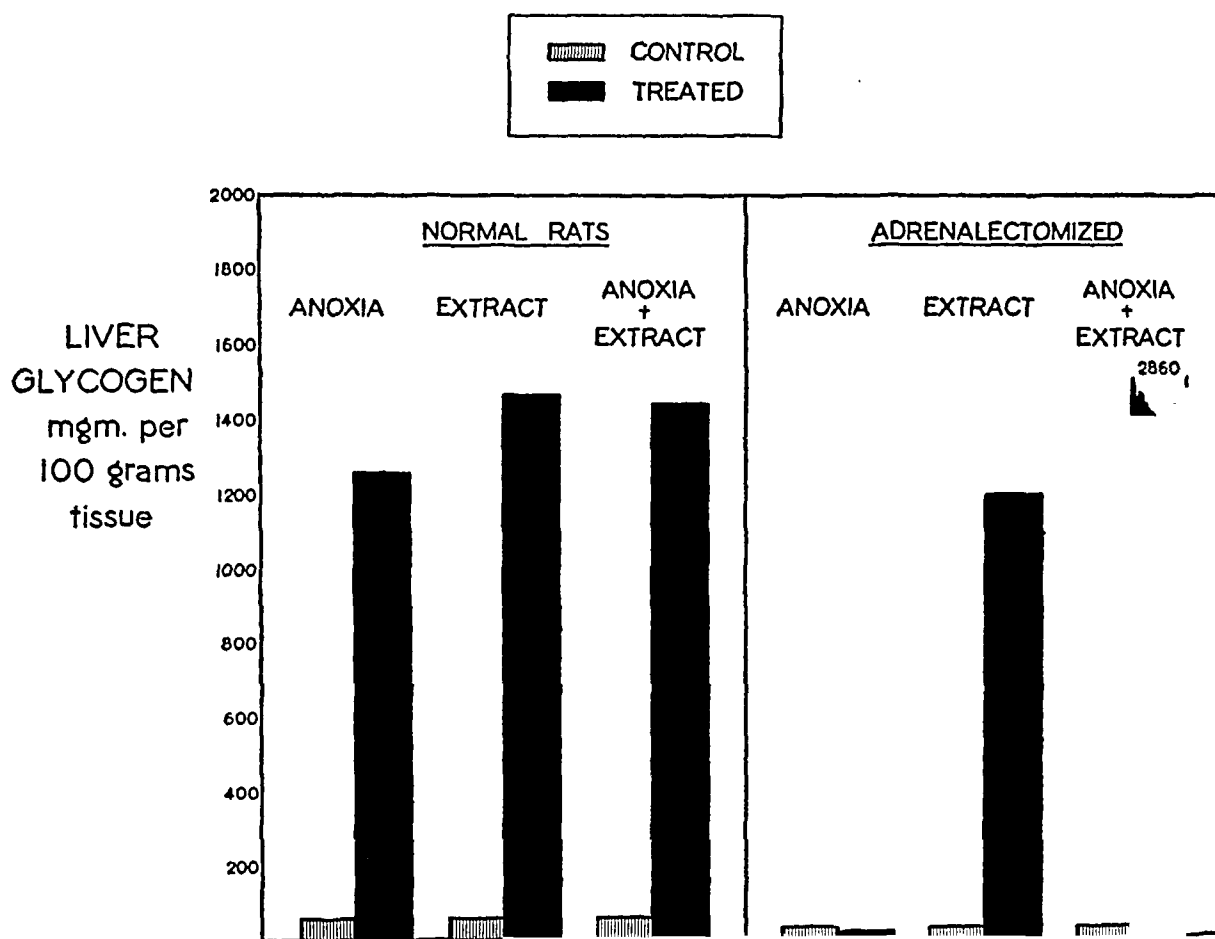


FIG. 4. EFFECT OF ANOXIA (24 HOURS AT 10.5 PER CENT O_2) AND ADRENAL CORTICAL EXTRACT TREATMENT ON LIVER GLYCOGEN

increase in carbohydrate stores that occurs after 24 hours of anoxia. A detailed study was made of the blood sugar, liver and muscle glycogen of rats exposed for 1.5, 3, 6, and 12 hours to reduced oxygen tension (59 mm. Hg partial pressure). In the early hours of exposure no significant change in blood sugar was observed. However, the *liver glycogen was considerably decreased during the early phase of exposure* when compared to the liver glycogen of normal rats fasted for a similar period (Figure 5).

An exposure period of 1.5 hours was selected for the study of the effect of extremely low oxygen tension on the blood sugar and liver glycogen of normal rats. The blood glucose during anoxia did not fall until the partial pressure of oxygen was reduced to 39 mm. Hg which was sufficient to reduce liver glycogen to a minimum (Figure 6). At this greatly reduced oxygen tension, fatal convulsions frequently occurred. However, very low liver glycogen was noted in animals that did not have convulsions.

8. Effect of anoxia (5 hours) on normal human subjects

Eight normal male subjects were exposed to an average oxygen tension of 98 mm. Hg after a 15-hour fast. Metabolic studies made during anoxia were compared to similar studies made during a comparable control period. All of the subjects exhibited most of the signs and symptoms which have been described in subjects exposed to low barometric pressure (Table VII). Severe frontal headache was noted in all but one case. Vasomotor collapse occurred in two subjects.

The significant metabolic changes are shown in Table VIII. Cyanosis was apparent in all cases, although the average arterial oxygen saturation was 83 per cent. Pulmonary ventilation was increased approximately 25 per cent. A definite decrease in hydrogen ion concentration was noted. Changes in oxygen consumption varied in different individuals but the average oxygen consumption of the group was increased about 5 per cent. Despite the fact that there was no decrease in the

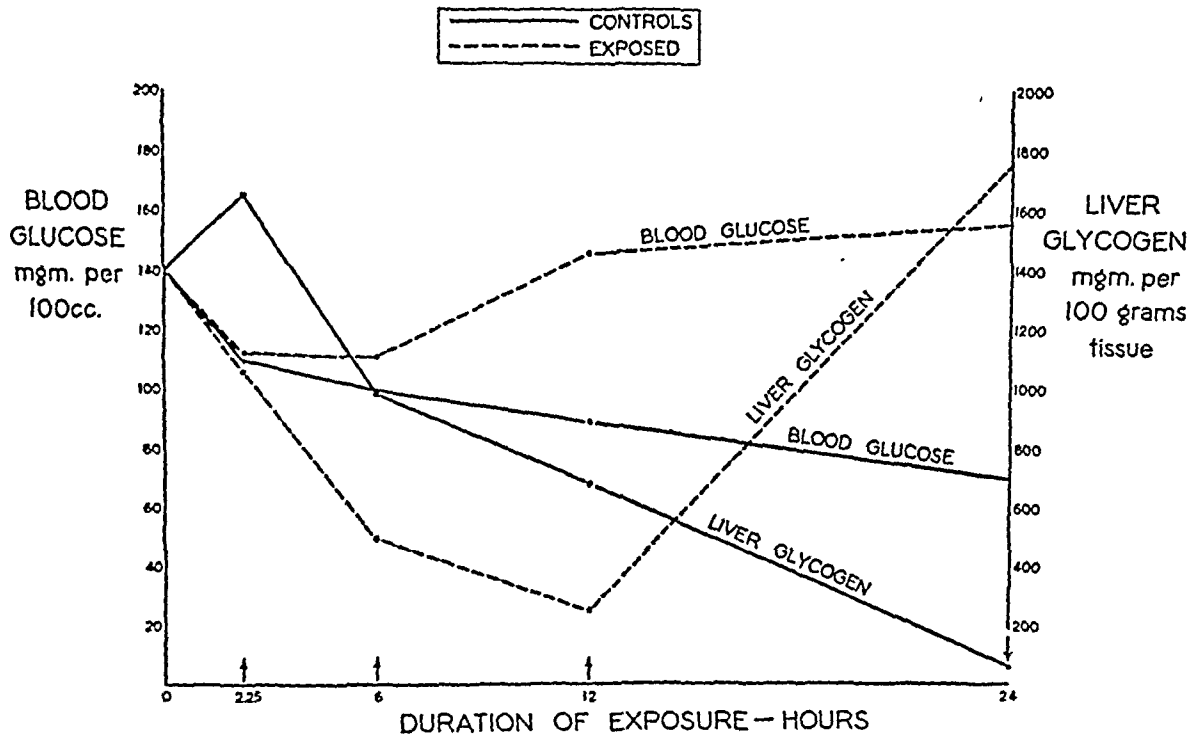


FIG. 5. THE EFFECT OF ANOXIA (7 PER CENT O_2) ON BLOOD GLUCOSE AND LIVER GLYCOGEN OF NORMAL RATS

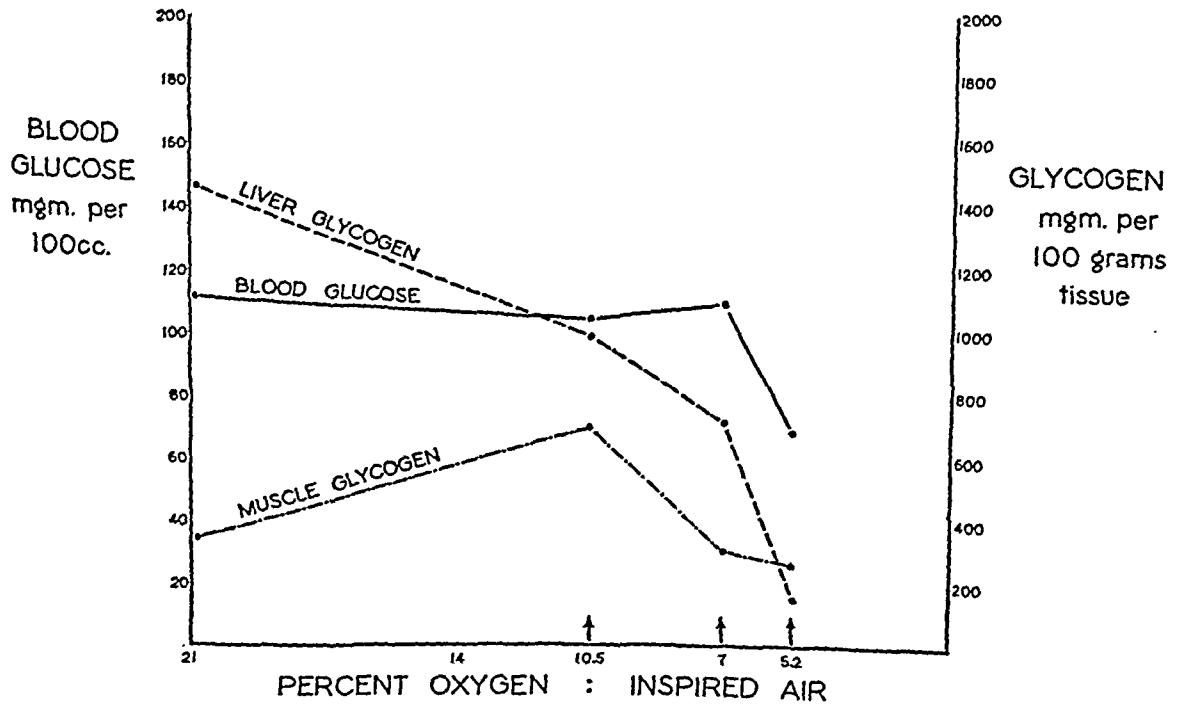


FIG. 6. THE EFFECT OF ANOXIA (1½ HOURS) ON BLOOD GLUCOSE, LIVER AND MUSCLE GLYCOGEN OF NORMAL RATS

TABLE VII

Effect on normal subjects of exposure to oxygen at a partial pressure of 98 mm. Hg for 5 hours

Subject	H.A.	S.D.	C.G.	R.G.	B.K.	G.K.	R.L.	G.T.	Summary
Oxygen partial pressure in mm. Hg.....	91	86	101	97	102	93	101	98	
Carbon dioxide partial pressure in mm. Hg.....	5	1	5	4	4	4	7	5	
Hyperventilation.....	++++	++++	+++	++	++	+++	++	++	8
Tachycardia.....	++	++	+	++++	++	++	+	+	8
Cyanosis.....	++	+++	++	++	+++	++	++	++	8
Frontal headache.....	+	+++	0	+++	++	+	++	++++	7
Mental exhilaration.....	+	0	+	++	0	+++	++	+++	6
General weakness.....	0	+++	0	0	++	0	0	+	3
Fall in blood pressure.....	0	0	0	0	++	0	0	++++	2
Syncope.....	0	0	0	0	+++	0	0	++++	2
Precordial discomfort.....	0	++	0	0	0	0	0	++	2
Nausea or vomiting.....	0	0	+	0	0	0	0	0	1

TABLE VIII

Metabolic changes in normal subjects exposed to oxygen at a partial pressure of 98 mm. Hg for 5 hours

	Control (8)	Anoxia (8)
Arterial oxygen saturation, per cent. .		83
Pulmonary ventilation, liters per hour	304	377
Respiratory rate, respirations per minute.....		16
Oxygen consumption, cc. per minute .	248	258
Respiratory quotient.....	78	81
Arterial pH.....	7.49	7.58
Serum chloride, m. eq. per liter.....	102.7	104.5
Serum sodium, m. eq. per liter.....	138.4	138.0
Serum potassium, m. eq. per liter....	5.6	5.4
Serum inorganic phosphorus, mgm. per 100 cc.....	3.9	2.9
Serum nonprotein nitrogen, mgm. per 100 cc.....		24
Blood sugar, mgm. per 100 cc.....	90	91
Urinary excretion of chloride, m. eq. per hour.....	6.5	5.9
Urinary excretion of sodium, m. eq. per hour.....	6.5	6.6
Urinary excretion of potassium, m. eq. per hour.....	3.8	3.7
Urinary excretion of phosphorus, grams per hour.....	0.023	0.012
Urinary excretion of nitrogen, grams per hour.....	0.600	0.518
Urinary excretion of water, cc. per hour.....	142	137

average oxygen consumption, there was a definite and consistent decrease in nitrogen excretion, without any increase in the serum nonprotein nitrogen. No significant change was noted in the fasting blood sugar, or in the curve following intravenous administration of glucose. Slight changes in the "T" waves of the electrocardiogram taken during anoxia were not prevented by the administration of glucose.

*Mineral metabolism**1. Effect of exposure for 24 hours to low oxygen tension*

In rabbits and monkeys the electrolyte concentrations in the blood of a control group of animals were compared with the concentrations in a group of animals exposed to low oxygen tensions for 24 hours. With dogs it was possible to obtain adequate blood samples from the same animal prior to and following anoxia and at the same time to determine the renal excretion of electrolytes.

A decrease in serum bicarbonate (2 to 5 m.eq. per liter) and an increase in serum chloride (4 to 8 m.eq. per liter) were noted following exposure to low oxygen tension for 24 hours. No significant change in the serum sodium or serum potassium was observed. When the exposure was associated with vomiting there was a rise in the nonprotein nitrogen. *During the 24-hour exposure the renal excretion of potassium, sodium and chloride of normal dogs was greatly increased* (Figure 7). A less marked increase in the excretion of inorganic phosphorus and total nitrogen was also noted. Similar but less exact changes were observed in the urine of normal rats exposed to low oxygen tensions for 24 hours.

2. Relation of the adrenal cortex to changes in mineral metabolism during exposure to low oxygen tension (80 mm. Hg) for 24 hours

It was possible to study the effect of anoxia upon two adrenalectomized dogs maintained with

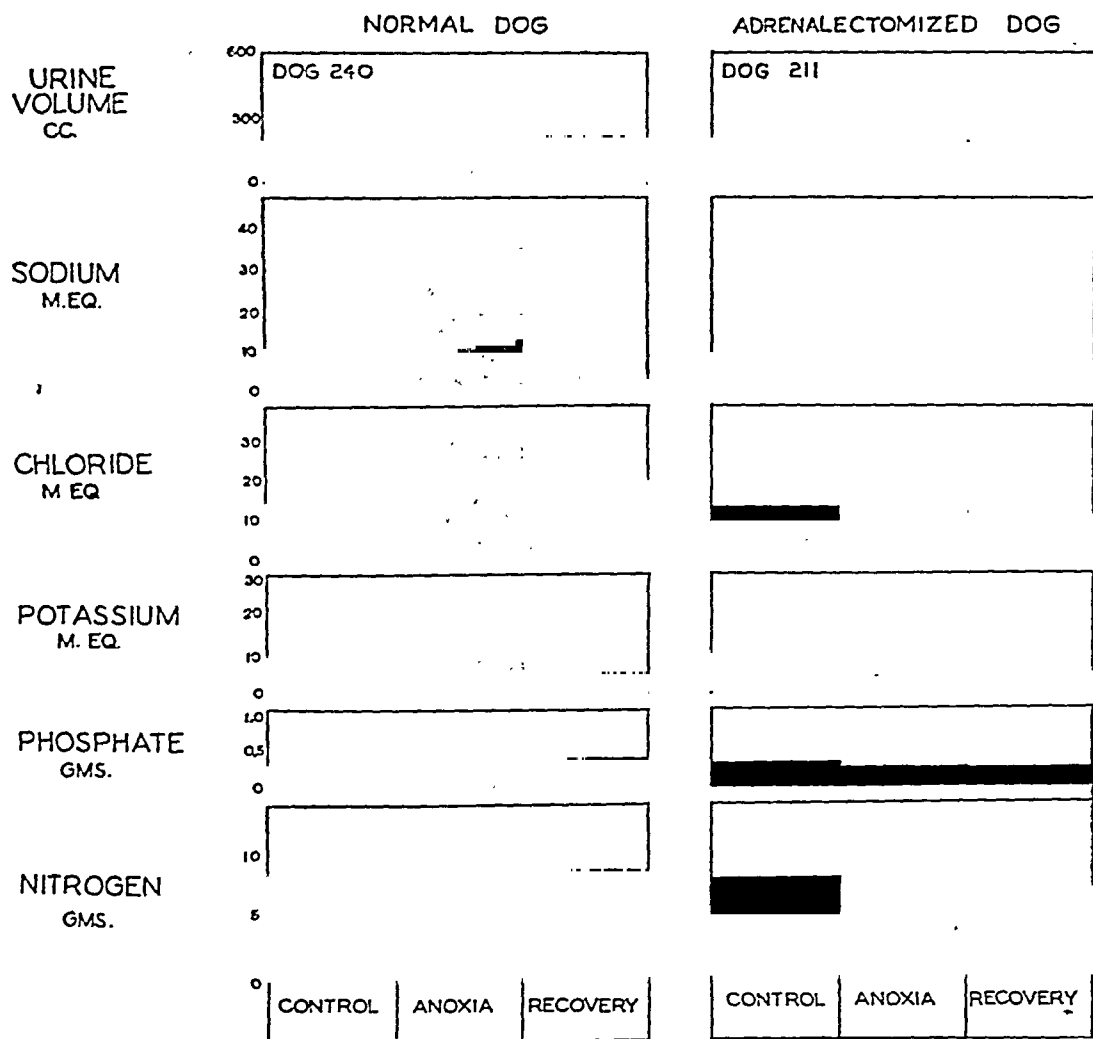


FIG. 7. THE RENAL EXCRETION OF NITROGEN AND ELECTROLYTES OF NORMAL AND ADRENALECTOMIZED DOGS BEFORE, DURING, AND AFTER EXPOSURE TO 10.5 PER CENT OXYGEN FOR 24 HOURS

desoxycorticosterone acetate and upon adrenalectomized rats maintained with sodium chloride or desoxycorticosterone. The changes in blood chemistry which occurred in the adrenalectomized dogs exposed to low oxygen tension were similar to the changes in blood chemistry that occurred in normal dogs during anoxia. There was also a striking increase in the renal excretion of potassium in adrenalectomized animals that resembled the effect of anoxia upon the excretion of potassium in normal animals. The slight increase in nitrogen and phosphorus excretion and the marked increase in sodium and chloride excretion that were noted in normal dogs failed to occur in adrenalectomized animals during exposure to anoxia. The failure

of adrenalectomized animals to excrete an increased quantity of nitrogen and phosphorus during anoxia is consistent with the observation that, in the absence of the adrenal cortex, protein catabolism does not increase during anoxia. The reason for the absence of any increase in the excretion of sodium and chloride, despite the large increase in the excretion of potassium, was not obvious. The failure of adrenalectomized animals to excrete excessive amounts of sodium and chloride during anoxia did not appear to be due to the maintenance dose of desoxycorticosterone acetate since adrenalectomized rats maintained on sodium chloride reacted in the same manner and since normal animals treated with desoxycorticosterone ace-

tate excreted large amounts of sodium and chloride during anoxia. The absence of any rise in the blood nonprotein nitrogen or serum potassium indicates that neither renal failure nor adrenal insufficiency was responsible for the failure of adrenalectomized animals to excrete increased quantities of sodium and chloride during anoxia.

3. *Effect of the "carbohydrate-regulating" factors of the adrenal cortex on the renal excretion of electrolytes at atmospheric oxygen tension*

From other studies (13) it was suspected that the increased secretion of adrenal cortical sub-

stances possessing "carbohydrate-regulating" activity might increase the excretion of sodium and chloride. The effect of treatment with 11-dehydro-17-hydroxycorticosterone on the renal excretion of normal and adrenalectomized animals was therefore studied (Figure 8). In the normal dog there was an increased excretion of nitrogen and phosphorus, and a more strikingly increased excretion of sodium, chloride and water. *The marked increase in the excretion of potassium which occurred in normal and adrenalectomized animals during anoxia did not occur during treatment with 11-dehydro-17-hydroxycorticosterone.* It was of interest to note that an adrenalectomized

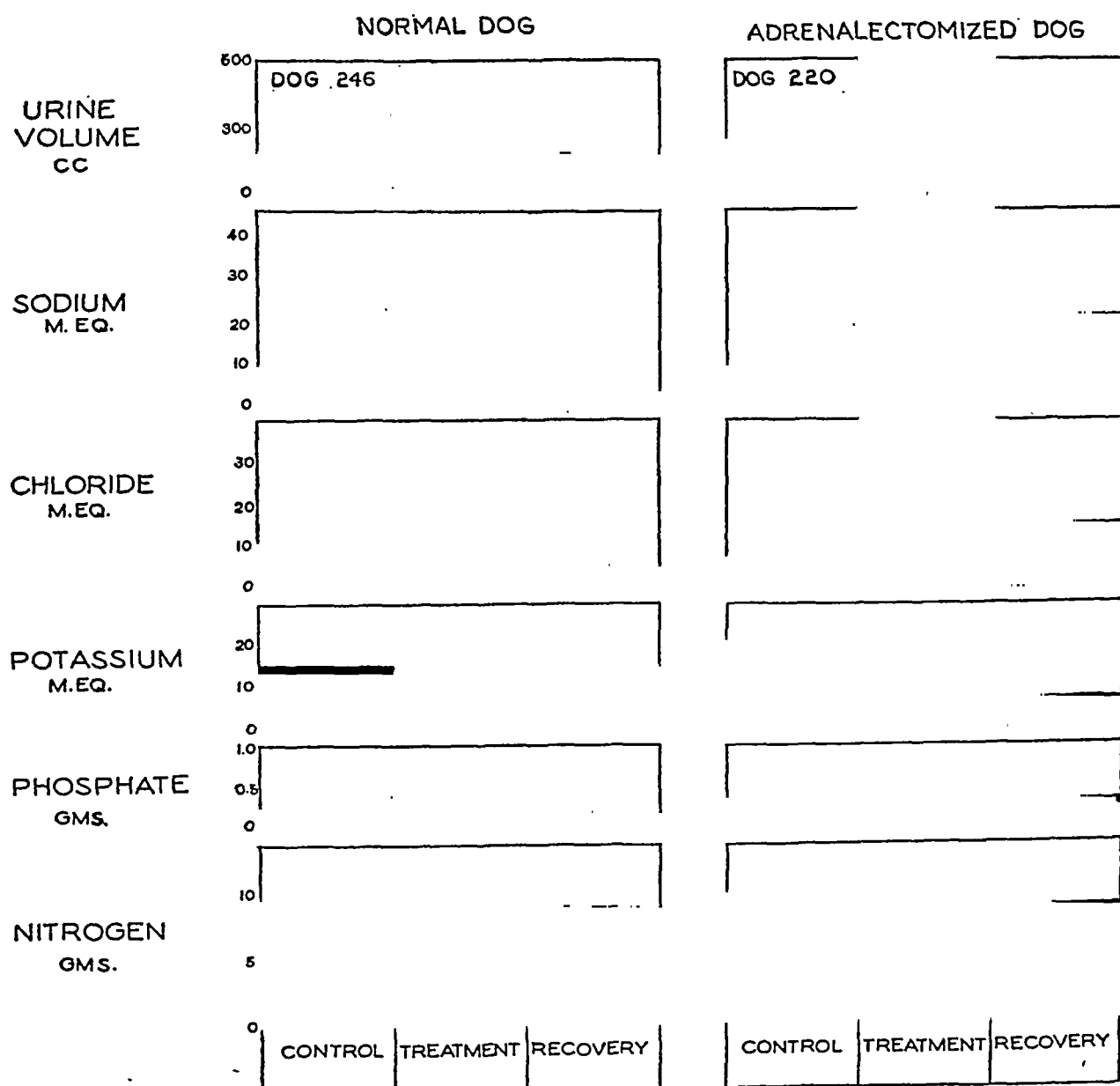


FIG. 8. THE RENAL EXCRETION OF NITROGEN AND ELECTROLYTES OF NORMAL AND ADRENALECTOMIZED DOGS BEFORE, DURING, AND AFTER TREATMENT WITH 11-DEHYDRO-17-HYDROXYCORTICOSTERONE

dog treated with 25 mgm. of 11-dehydro-17-hydroxycorticosterone showed the same changes in sodium, chloride, phosphorus and nitrogen excretion that were observed in normal animals during anoxia. The serum concentration of sodium, potassium, chloride and carbon dioxide was not affected by treatment with 11-dehydro-17-hydroxycorticosterone, although a rise in nonprotein nitrogen occurred.

4. Effect of anoxia (5 hours) on the mineral metabolism of human subjects

Changes in the serum concentration and renal excretion of electrolytes were studied in eight normal subjects exposed to an average oxygen tension of 98 mm. Hg (Table VIII). There was a distinct reduction in hydrogen ion concentration. There was no change in the renal excretion of sodium and potassium, although the nonprotein nitrogen and phosphorus excretion were slightly reduced.

In contrast to the increase in nitrogen and phosphorus excretion which was observed during the 24-hour period of anoxia in animal experiments, the excretion of these substances by human subjects during a 5-hour period of anoxia was decreased.

DISCUSSION

The observations which compose this report were made by exposing experimental animals and human subjects to an atmosphere of reduced oxygen concentration at normal atmospheric pressure. No effort was made to simulate the changes in total pressure which occur at high altitudes. It is known, however, that the same degree of arterial oxygen unsaturation occurs at moderately reduced oxygen tension whether this is due to low oxygen concentration or low total pressure (14). At greatly reduced pressures a difference in gaseous exchange, due to the increase in the mean free path of the gas molecules, may occur (15).

The difference between liver glycogen of rats exposed to low oxygen tension and liver glycogen of control rats was larger than the difference noted in other species. This is due to the fact that, whereas the liver glycogen of rats fasted for 24 hours is uniformly almost negligible, in other species it is impossible to effect a total depletion of liver glycogen by starvation for 24 hours.

When dogs were exposed to low oxygen tension or treated with adrenal cortical extract neither blood glucose nor liver glycogen rose significantly. This species peculiarity may be due to the fact that dogs were fed a diet composed almost exclusively of protein and fat. Thus, whether dogs were fed, fasted, exposed to low oxygen tension, or treated with adrenal cortical extract, the same type of foodstuff was being utilized. A similar phenomenon has been described in rats maintained on a diet rich in protein (16).

A delay in the appearance of increased nitrogen excretion which occurs during anoxia was described by Brunquist *et al.* (17). This observation is consistent with the fact that protein catabolism is diminished during the early phase of anoxia and increased only in the later phase of anoxia. Furthermore, in the absence of the adrenal cortex, no increase in protein catabolism occurs and the nitrogen excretion during the entire 24-hour period of anoxia is less than during a similar control period.

The depletion of liver glycogen that occurs during the initial phase of anoxia was described by Paul Bert in 1878: "quand la depression est forte et qu'elle agit pendant longtemps (5 to 6 hours) le sucre diminue plus ou moins dans le foie" (18). During exposures to moderately reduced oxygen tension blood glucose is maintained at a normal level at the expense of liver glycogen. During exposures to greatly reduced oxygen tension a fall in blood glucose occurs after the liver glycogen has been exhausted. These experiments indicate that the primary effect of anoxia is exactly the opposite of the effect of adrenal cortical "carbohydrate-regulating" hormone, although the effect upon liver glycogen of a 24-hour exposure to low oxygen tension is similar to the effect of treatment with this hormone. In adrenalectomized animals the primary effect of anoxia continues throughout the exposure and the animals frequently succumb with hypoglycemic convulsions. In normal animals increased utilization of carbohydrate may continue throughout the period of exposure, but when glycogen is exhausted protein sources come to the rescue and in the end there is an accumulation of glycogen stores.

Although most of the studies here reported were made at oxygen tensions which correspond to 18,000 feet, it is interesting to note that definite

changes in carbohydrate metabolism were noted at oxygen tensions that correspond to 11,000 feet. It was not feasible to expose human subjects to anoxia for 24 hours. However, the blood glucose of monkeys increased during a 24-hour exposure to anoxia. This elevation of blood glucose is similar to that which occurs in patients suffering from the anoxemia of carbon monoxide poisoning (19). Depletion of liver glycogen probably accounts for the increased susceptibility of animals to insulin during the early phase of exposure to low oxygen tension (20).

The reactions of human subjects to low oxygen concentration were similar to the reactions which occur during exposure to low barometric pressure. Two subjects had a momentary lapse of consciousness toward the end of the 5-hour exposure, one after an unsuccessful arterial puncture. The syncope, characterized by a slowing of the pulse and a fall in blood pressure, was followed by a rise in blood glucose and serum nonprotein nitrogen. However, both of the individuals who experienced these attacks had also experienced similar reactions while on automobile or boat trips. Although both of the subjects who fainted were in the group of four that were not given intravenous glucose, it was not thought that this was of particular significance because the administration of glucose was not associated with improvement in subjective sensations. Electrocardiographic changes during anoxia were not restored to normal by the administration of glucose, although changes in the electrocardiogram during anoxia have been correlated with abnormalities of carbohydrate metabolism (21). The reduction in the hydrogen ion concentration of the arterial blood may have been due to the fact that human subjects were kept in bed under basal conditions throughout the exposure periods.

Changes in oxygen consumption were too small to be of significance. On the other hand, despite the absence of a fall in the oxygen consumption, a consistent fall in nitrogen excretion was observed. No increase in the serum nonprotein nitrogen occurred, suggesting that during the early phase of anoxia in human subjects there is a shift of metabolism from protein to carbohydrate similar to that demonstrated in experimental animals.

There are few data available relative to the

effect of low oxygen tension upon the excretion of electrolytes with the exception of Sundstroem's observations (22) of subjects on mountain expeditions. A consistent increase in potassium excretion was found in all animals, normal or adrenalectomized, that were exposed to low oxygen tension for 24 hours. Part of the increased excretion of potassium during exposure to low oxygen tension may be associated with the rapid loss of glycogen from the liver which occurs initially in normal and adrenalectomized animals. It appears that the increased renal excretion of potassium which occurs during exposure to anoxia represents for the most part a direct effect of anoxemia and not an effect mediated by the adrenal cortex inasmuch as it occurs in both normal and adrenalectomized animals exposed to anoxia and since little or no increase in potassium excretion followed the administration of adrenal cortical "carbohydrate-regulating" factor.

The fact that normal animals exposed to low oxygen tension for 24 hours excreted large quantities of sodium and chloride, whereas adrenalectomized animals similarly exposed did not excrete an increased quantity of sodium and chloride, was thought at first to be due to differences in the respiratory response of these animals. Cope has demonstrated such a difference (23) and Hoffman *et al.* (24) have reported an unusual encephalo-electrical sensitivity to hyperventilation in the majority of a group of twenty-five patients with Addison's disease. However, the magnitude of the shift in serum bicarbonate and chloride ions during anoxia was of the same order of magnitude in normal and adrenalectomized animals.

From the observations of Ingle and Thorn (13) who compared the effect of desoxycorticosterone acetate and 11-dehydro-17-hydroxycorticosterone in partially depancreatized rats, it was suspected that an increase in sodium and chloride excretion might be produced by adrenal cortical "carbohydrate-regulating" hormone. Recent studies (25) confirm the fact that treatment with certain adrenal cortical steroid compounds which possess a hydroxyl group on C₁₇ induces sodium and chloride excretion in contrast to the well-known "sodium-retaining" effect of such adrenal cortical steroid compounds as corticosterone and 11-desoxycorticosterone. In contrast to the retention of water that occurs during treatment with "so-

dium-retaining" sterols, there was a considerable increase in the excretion of water during treatment with "carbohydrate-regulating" factor. The increase in sodium and chloride excretion that was observed was greater than the increase in water excretion, but the concentration of sodium and chloride excreted bears a close relation to the concentration of these substances in the body fluids. The excretion of chloride is small in proportion to that of sodium, possibly because chloride is retained to replace carbon dioxide lost through overventilation.

It appears consistent that during anoxia adrenalectomized animals which are unable to increase protein catabolism by increasing the secretion of adrenal cortical "carbohydrate-regulating" factor do not exhibit the increase in sodium and chloride excretion that this hormone induces.

There is no evidence that the effect of adrenal cortical "carbohydrate-regulating" factor in increasing the excretion of sodium and chloride during anoxia is beneficial, whereas its effect in increasing protein catabolism might be a favorable reaction since during exposure to low oxygen tension there is an increased utilization of carbohydrate. Furthermore, the work of McFarland and Forbes (26) suggests that an increase in carbohydrate may alleviate to some extent the deleterious effects of anoxemia upon higher functions.

SUMMARY

1. Normal human subjects exposed to an average oxygen tension of 98 mm. Hg for 5 hours had an average arterial oxygen saturation of 83 per cent. Despite the fact that there was no decrease in the oxygen consumption there was a definite and consistent decrease in the nitrogen excretion.

2. Experimental animals exposed to low oxygen tensions for similar periods showed a depletion of liver glycogen that was not associated with a fall in blood glucose level unless the anoxia was very severe.

3. Exposure of normal animals to low oxygen tension for 24 hours was associated with a rise in blood glucose and liver glycogen (except in dogs), and an increase in the renal excretion of nitrogen (in all animals).

4. Adrenalectomized animals succumbed when exposed to oxygen tensions that normal animals

were able to withstand, and no increase in blood glucose, liver glycogen or nitrogen excretion occurred.

5. Treatment with "carbohydrate-regulating" factor of the adrenal cortex enabled adrenalectomized animals to withstand exposure to an otherwise fatal oxygen tension, and resulted in an increase in blood glucose, liver glycogen and nitrogen excretion.

6. Normal animals exposed to low oxygen tensions for 24 hours showed a slight increase in nitrogen and phosphorus excretion and a marked increase in sodium, chloride and potassium excretion.

7. Adrenalectomized animals exposed to low oxygen tensions for 24 hours exhibited a striking increase in potassium excretion but no increase in nitrogen, phosphorus, sodium or chloride excretion.

8. Treatment of normal and adrenalectomized animals with "carbohydrate-regulating" factor of the adrenal cortex was followed by a striking increase in sodium, chloride and water excretion but no significant increase in potassium excretion.

CONCLUSION

During the initial phase of anoxia there appears to be an increased utilization of carbohydrate. A normal blood glucose level is maintained at the expense of liver glycogen stores. Successful adaptation to continued exposure to low oxygen tension depends in part upon an increase in protein catabolism with a subsequent rise in carbohydrate stores and increase in nitrogen excretion. These changes do not occur in the absence of the adrenal cortex. Acute anoxia is accompanied by a rise in the chloride ion concentration of the serum and by a fall in the hydrogen ion concentration (rise in pH) of the blood. Prolonged anoxia (24 hours) leads to a marked increase in the renal excretion of sodium, chloride and potassium. The increase in potassium excretion appears to be accounted for principally by factors other than the adrenal cortex, whereas the increase in sodium and chloride excretion appears to be mediated by the "carbohydrate-regulating" factor of the adrenal cortex.

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BIBLIOGRAPHY

1. Evans, G., The effect of low atmospheric pressure on the glycogen content of the rat. *Am. J. Physiol.*, 1934, 110, 273.
2. Evans, G., The adrenal cortex and endogenous carbohydrate formation. *Am. J. Physiol.*, 1936, 114, 297.
3. Armstrong, H. G., and Heim, J. W., Effect of repeated daily exposures to anoxemia. *J. Aviation Med.*, 1938, 9, 92.
4. Armstrong, H. G., Principles and Practice of Aviation Medicine. Williams and Wilkins Co., Baltimore, 1939, pp. 286-288.
5. Lewis, R. A., and Koepf, G. F., An apparatus for producing constant gas mixtures. *Science*, 1941, 93, 407.
6. Folin, O., and Malmros, H., An improved form of Folin's micro method for blood sugar determinations. *J. Biol. Chem.*, 1929, 83, 115.
7. Good, C. A., Kramer, H., and Somogyi, M., The determination of glycogen. *J. Biol. Chem.*, 1933, 100, 485.
8. Thorn, G. W., Engel, L. L., and Eisenberg, H., The effect of corticosterone and related compounds on the renal excretion of electrolytes. *J. Exper. Med.*, 1938, 68, 161.
9. Thorn, G. W., Engel, L. L., and Eisenberg, H., Treatment of adrenal insufficiency by means of subcutaneous implants of desoxycorticosterone acetate. *Bull. Johns Hopkins Hosp.*, 1939, 64, 155.
10. Thorn, G. W., Howard, R. P., and Emerson, K., Jr., Treatment of Addison's disease with desoxycorticosterone acetate. *J. Clin. Invest.*, 1939, 18, 449.
11. McFarland, R. A., and Dill, D. B., A comparative study of the effects of reduced oxygen pressure on man during acclimatization. *J. Aviation Med.*, 1938, 9, 18.
12. Long, C. N. H., Katzin, B., and Fry, E. G., The adrenal cortex and carbohydrate metabolism. *Endocrinology*, 1940, 26, 309.
13. Ingle, D. J., and Thorn, G. W., A comparison of the effects of 11-desoxycorticosterone acetate and 17-hydroxy-11-dehydrocorticosterone in partially depancreatized rats. *Am. J. Physiol.*, 1941, 132, 670.
14. Dill, D. B., Edwards, H. T., and Robinson, S., Pulmonary gaseous exchanges at low barometric pressure and in air mixed with nitrogen. *J. Aviation Med.*, 1939, 10, 3.
15. Haldane, J. S., and Priestley, J. G., *Respiration*. Second Edition. Yale University Press, New Haven, 1935, p. 301.
16. Mirski, A., Rosenbaum, I., Stein, L., and Wertheimer, E., On the behaviour of glycogen after diets rich in protein and in carbohydrate. *J. Physiol.*, 1938, 92, 48.
17. Brunquist, E. H., Schneller, E. J., and Loevenhart, A. S., The effects of anoxemia on nitrogen metabolism. *J. Biol. Chem.*, 1924, 62, 93.
18. Bert, P., *La Pression Barometrique*. G. Masson, Paris, 1878.
19. Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry, Volume I, Interpretations*. Williams and Wilkins Co., Baltimore, 1931, p. 191.
20. Glickman, N., and Gellhorn, E., The effect of oxygen deficiency on the sensitivity of rats to insulin. *Am. J. Physiol.*, 1938, 121, 358.
21. Larsen, K., Effect of anoxemia on the human electrocardiogram. *Acta. med. Scandinav.*, supp. 1936, 78, 141.
22. Sundstroem, E. S., Studies on adaptation of man to high altitudes, V. Univ. California Publications *Physiol.*, 1919, 5, 121.
23. Cope, O., Personal communication.
24. Hoffman, W. C., Lewis, R. A., and Thorn, G. W., The electroencephalogram in Addison's disease. (To be published.)
25. Thorn, G. W., Engel, L. L., and Lewis, R. A., The effect of 17-hydroxycorticosterone and related adrenal cortical steroids on sodium and chloride excretion. *Science*, 1941, 94, 348.
26. McFarland, R. A., and Forbes, W. H., The effects of variations in the concentration of oxygen and of glucose on dark adaptation. *J. General Physiol.*, 1940, 24, 69.

EFFECTS OF INTERRUPTING AND RESTORING THE CIRCULATION TO THE LOWER EXTREMITIES¹

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It has been established that the heart accelerates on release of tourniquets previously applied to the extremities (2 to 9, 13). The mechanism for this acceleration has not been adequately explained.

Jarisch and Gaisböck (12) have postulated a reflex origin of this acceleration, but offered no supporting evidence. They anticipated a blood pressure fall simultaneous with the rise in heart rate but obtained no evidence of the former with the auscultatory method. Asmussen, Christensen and Nielsen (5, 13) noted that the cardiac acceleration was greatest when the subject was in the upright posture and least in the head-down position. The release of the tourniquet, they found, produced a drop in both systolic and diastolic pressure, the magnitude of the pressure drop likewise varying with posture. They considered the acceleration a reflex compensatory to the blood pressure fall, and they attributed the latter to stimulation of "pressor-sensitive zones" assumed to exist in the large limb arteries. Alam and Smirk (1, 2) attributed the cardiac acceleration to the release into the blood stream of metabolites arising from the previously occluded extremities.

In order to study this phenomenon further, a series of experiments was carried out in which the relation of the cardiac acceleration to changes in accurately recorded arterial and venous pressure was determined. The influence of altering several variables on the magnitude of cardiac acceleration was also determined. In the course of this study, opportunity was afforded for measuring some of these circulatory changes occurring on application of and during arterial and venous occlusion as well as following their release. These are described in the report and their bearing on the major purpose of the presentation is indicated.

METHOD

Thirty-one subjects were studied. Twenty-seven were selected as normal young adults (physicians, technicians

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and convalescent surgical patients) from 16 to 35 years of age; twenty-five of them were males and two females. The other four subjects had moderately advanced thromboangiitis obliterans and ranged in age from 32 to 52 years.

All observations were made on recumbent subjects after a rest period of 15 to 30 minutes in a warm, quiet and darkened room. While the conditions were not basal, they were sufficiently stable to eliminate most of the extraneous factors which might influence heart rate, as is demonstrated by the small magnitude of the spontaneous pulse rate changes in the control period (Table I). At the beginning of the rest period, electrodes lead-

TABLE I
Changes in heart rate on release of bilateral arterial occlusion

Subject	Maximum resting variation	Maximum rise on release	Time of onset of acceleration		Time of peak of acceleration		Time of return to resting level	
			Beat	Second	Beat	Second	Beat	Second
	beats per minute	beats per minute						
MF	10	18	1	0.8	10	7	ca. 115	75
MF	11	15	1	0.8	8	6	>29	>20
MF	8	35	1	1.0	7	5	18	13.5
MF	7	29	2	0.9	7	5	16	12
EK	15	25	2	0.8	11	7.5	20	13.5
EK	13	15	2	0.9	6	5.9	20	20
EK	14	13	5	3.1	11	6.8	21	15
Ca	4	13	3	1.5	10	5	67	35
Ca	4	17	5	2.8	26	15	48	27
G	7	25	3	2.1	12	7	31	17.5
Ka.	10	14	2	1.0	7	5	13	8
Br	10	>20	2	1	7	5	31	22
Cr	10	20	1	1.1	14	12	19	15
Average	9.5	20	2.3	1.4	10.5	6.3	28	23

ing to an electrocardiograph were strapped on the wrists, and wide leather-backed pneumatic cuffs were placed about both upper thighs. Abrupt inflation of these cuffs was achieved by suddenly connecting them to a pressure tank previously set at the desired pressure level. This avoided congestion of the legs. Such occlusion was usually unaccompanied by pain even when pressures of 300 mm. Hg were used. Deflation of the cuffs was likewise brisk. In all new subjects, trials were carried out to familiarize them with the sensations involved in the procedure.

Pressure over 200 mm. Hg was used to ensure almost complete arterial obstruction (19). The only sensation

noted during the period of occlusion was a feeling of warmth in the limbs, which increased to an unpleasant sense of heat when the constriction was prolonged. During the occlusions, which were rarely over 15 minutes in duration, several of the subjects actually dozed. On restoration of blood flow, a transient sensation of coolness was experienced, succeeded by a wave of warmth flowing down the limbs and then by tingling and numbness. These subjective phenomena were accompanied by visible hyperemia.

In the preliminary experiments, the pulse rate was measured by palpating the radial artery for 30-second periods timed by a stopwatch. In the main group of experiments, the electrocardiograph, with the camera speed reduced to 6 mm. per second, was used to record heart rate. Further, in a group of 13 tests, the ordinary camera speed of 25 mm. per second was used in order to follow instantaneous heart rate from beat to beat (60/individual cycle length in seconds). For the most part heart rate was recorded for the first 15 seconds of each minute during the rest periods and during maintained occlusion, and the rate per minute calculated by multiplying by 4 the number of beats and fractions thereof in this 15-second interval. Recording was begun 30 seconds before and continued until 45 seconds after the act of application or release of occlusion. The rate per minute was arrived at as above, except in those experiments in which the cycle length from beat to beat was measured. At least 5 minutes were allowed between successive occlusions to permit the heart rate to return to its resting level.

In 6 preliminary observations, blood pressure was measured by the auscultatory method. In the remainder (7 experiments) the brachial pressure was recorded optically by intraarterial puncture with the Hamilton needle manometer (10). Femoral venous pressure was also recorded by an optical manometer (15).

RESULTS

1. *The cardiac acceleration on release of arterial occlusion of the limbs.* The data from 13 experi-

ments on seven normal subjects in which the heart rate was measured cycle by cycle is assembled in Table I and typical responses are shown in Figures 1 and 5. A transitory increase in heart rate occurred uniformly on release of occlusion. It was longer in duration and greater than the variation in rate in the control periods which results from sinus arrhythmia.

In 52 bilateral arterial occlusions in four normal subjects, average rates measured over 15-second intervals were calculated. It was found that the average cardiac acceleration on release was 15 beats per minute for an average duration of 1.5 minutes. In 26 other observations in which heart rate was measured by radial artery palpation, the cardiac acceleration on release averaged 11 beats per minute.

2. *The blood pressure changes on release of arterial occlusion of the limbs.* The data of 7 experiments on four normal subjects in whom intraarterial pressure was recorded on release of occlusion are shown in Table II, and Figure 1 illustrates the time course in a typical experiment. A precipitous blood pressure fall at the instant of cuff deflation occurred in every instance, with one exception in which the drop was delayed 1 second. Systolic and diastolic pressure changes were parallel in all instances. The magnitude of these drops was significantly greater than the maximum resting variation in blood pressure in any given case. The fall in pressure increased with the duration of occlusion (*cf.* Table II).

In 5 of the 7 experiments listed in Table II, measurements were made of the instantaneous

TABLE II
Effect on brachial intraarterial pressure and heart rate of release of bilateral arterial occlusion

Subject	Duration of occlusion	Maximum blood pressure variation at rest*	Maximum systolic pressure decrease on release	Maximum diastolic pressure decrease on release	Maximum decrease in diastolic notch level on release	Duration of blood pressure drop	Time of onset of blood pressure drop	Time of onset of pulse acceleration
	minutes	mm. Hg	mm. Hg	mm. Hg	mm. Hg	seconds	seconds	seconds
EK	1	6	10	11	20	12	Instantaneous	0.9
EK	2	7	11	10	20	11	Instantaneous	3.1
EK	6	8	14	10	25	30	Instantaneous	0.8
G	7	4	16	13	30	15	1	2.1
Ka.	15	5	17	14	29	>20	Instantaneous	1
Kr.	16	15	27	14	?	>10	Instantaneous	?
Kr.	21	20	38	27	25	>25	Instantaneous	?
Average		9	19	14	25	18		1.6

* Systolic or diastolic.

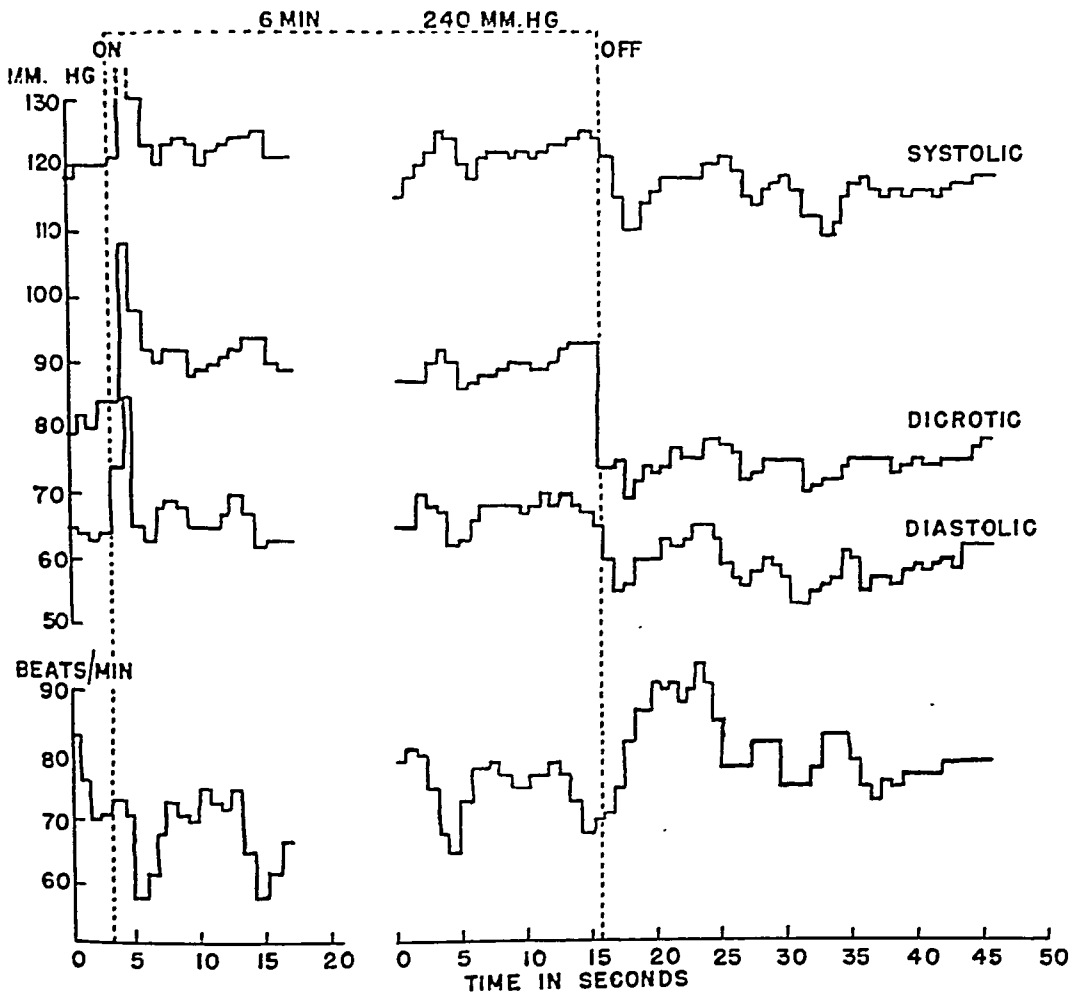


FIG. 1. A TYPICAL RESPONSE IN BLOOD PRESSURE AND HEART RATE ON APPLICATION AND ON RELEASE OF BILATERAL ARTERIAL OCCLUSION OF THE LOWER EXTREMITIES IN A NORMAL SUBJECT

Blood pressure was recorded with the Hamilton needle manometer in the brachial artery, and heart rate recorded with the electrocardiograph at camera speed of 2.5 cm. per second.

heart rate changes and in every instance the cardiac acceleration did not occur until 0.8 to 3.1 seconds after the blood pressure fell.

Simultaneously with the pressure drop, the pulse contour changed, reflecting the lowering of peripheral resistance (Figure 2). The change consisted in a lowering of the level of the dicrotic notch, an increased celerity to the early part of the catacrotus and a smoothing out of the summit of the curve. The drop in level of the dicrotic notch was greater than that of the systolic and diastolic pressures (*cf.* Figure 2 and Table II).

3. *The relation of the acceleration on release to the duration of the previous occlusion of the limbs.* The degree of acceleration of the heart rate varied

directly with the duration of prior occlusion. This relationship is summarized in Figure 3 which presents the total rise in heart rate. The rough proportionality between the cardiac acceleration on release and the duration of prior occlusion applied for periods of occlusion of from 3 to 15 minutes is made more evident by considering the integrated increase in rate than from observation of either maximum increase or duration of increase alone. Short occlusions of $\frac{1}{4}$ to 2 minutes were followed by pulse accelerations, but the magnitude was within the range of resting variation. They were, however, consistent in occurrence and persisted longer than the resting variations, suggesting that they were significant. Oc-

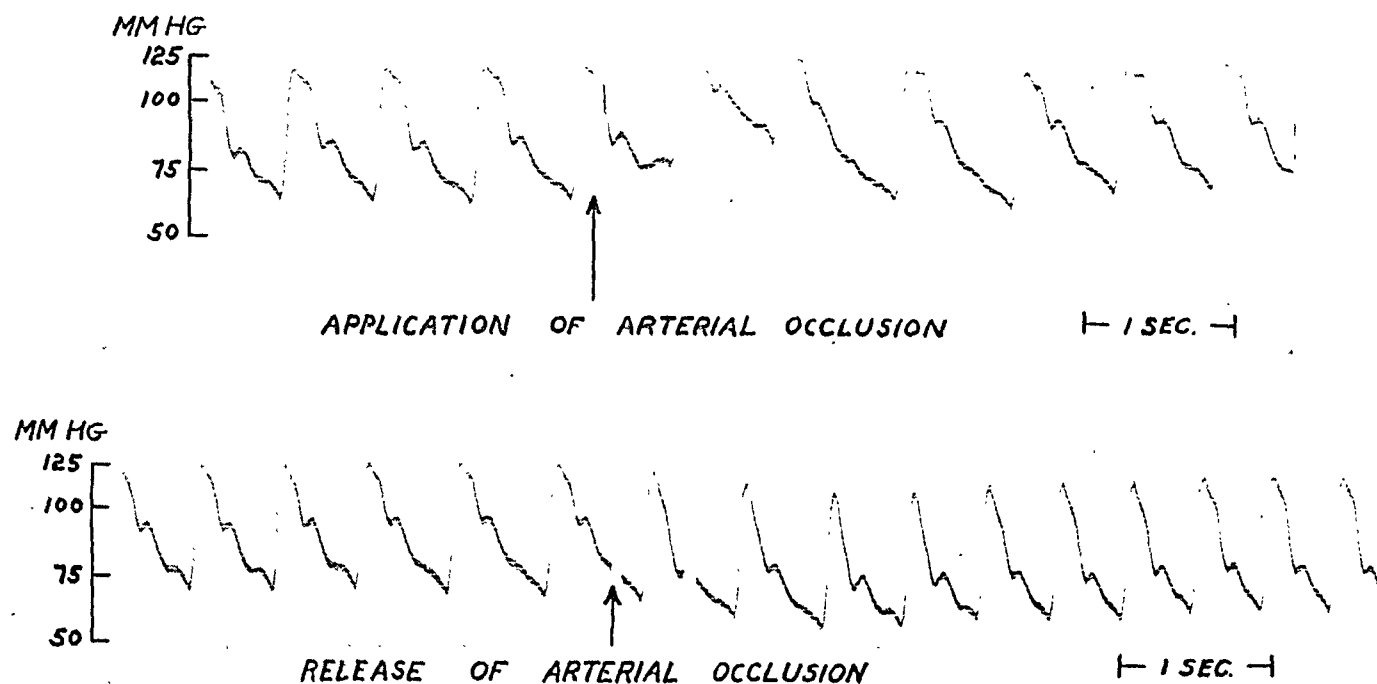


FIG. 2. A TYPICAL RECORD OF BLOOD PRESSURE IN THE BRACHIAL ARTERY ON APPLICATION AND ON RELEASE OF BILATERAL ARTERIAL OCCLUSION IN THE NORMAL SUBJECT, RECORDED WITH HAMILTON NEEDLE MANOMETER

The arrows point to the interruption in the beam which signaled the instant of application and release.

clusions of over 15 minutes produced pain and thereby an acceleration in heart rate during the later minutes of occlusion, masking the acceleration following release of occlusion.

4. *The changes in venous pressure in the femoral vein following release of the arterial occlusion, and their time relation to the cardiac acceleration.* In two trained subjects 4 observations were made by optically recording the femoral venous pressure during and after release of arterial occlusion. A typical result is shown in Figure 4 and the data are summarized in Table III. During arterial occlusion the femoral venous pressure fell. After a lag of 3 to 9 seconds following release, there was a slight rise, reaching a maximum value, approximately at the resting level, within 13 to 40 seconds.

TABLE III

Femoral venous pressure upon release of arterial occlusion

Subject	Initial resting femoral venous pressure	Pressure at end of occlusion period	Maximum pressure after release	Time when rise begins	Time of maximum pressure
	cm. H ₂ O	cm. H ₂ O	cm. H ₂ O	seconds	seconds
CR	5.5	3.8	5.3	9	40
CR	5.3	2.5	3.3	8	>28
CA	11.5	10.0	11.8	3	13
CA	11.5	11.0	12.2	5	14

TABLE IV

Effect of release of arterial occlusion (maintained for 3 to 15 minutes) in thromboangiitis obliterans

Subject	Severity of disease	Maximum heart rate variation at rest	Change in heart rate on release of occlusion of both legs	Change in heart rate on release of occlusion of worse leg only
		beats per minute	beats per minute	beats per minute
SC	+	8	+ 5 - 4, +8†	-3, +7
HS	++	4	+10 + 6	+6
ML	+++	6	+ 3	+4 +4* +4* -7* -3*
MA	++++	7	+ 7 - 4 - 9* -10* - 2, +4† - 2, +4† - 3, +6†	+5* 0* -6*

* Pulse rate measured by counting radial pulse for 10-second periods timed by stopwatch. All other measurements made on electrocardiograph by usual method except in cases where instantaneous pulse rate was measured.

† Instantaneous pulse rate measured.

5. *The effect of release of bilateral arterial occlusion on the heart rate in patients with thrombo-*

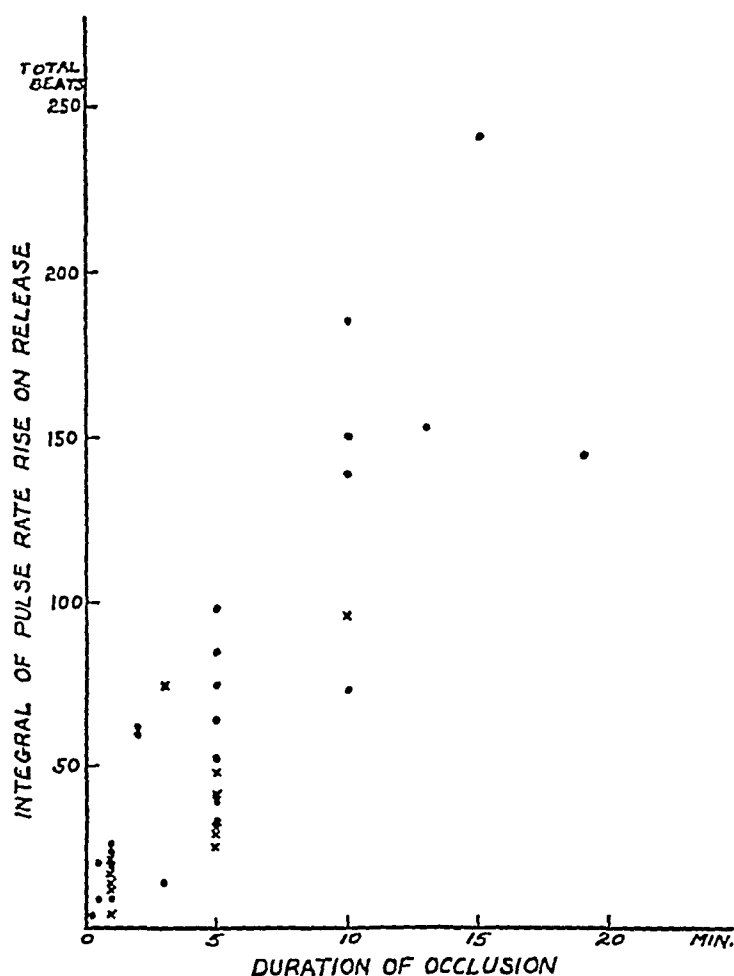


FIG. 3. THE EFFECT OF DURATION OF PRIOR OCCLUSION ON THE TOTAL RISE IN HEART RATE (INTEGRAL OF RISE) FOLLOWING RELEASE OF BILATERAL ARTERIAL OCCLUSION OF THE LOWER EXTREMITIES IN THE NORMAL SUBJECT

Heart rate was recorded with the electrocardiograph at camera speed of 6 mm. per second. Solid circles designate one subject and crosses a second subject.

angiitis obliterans. The effect of release of arterial occlusion was tested in four patients with moderately advanced thromboangiitis obliterans and the results are summarized in Table IV. Insignificant acceleration occurred when occlusion of the limbs was released, and any slight rise in heart rate tended to occur later than in the normal. The acceleration was greater than the resting variation in only one patient, H. S., who had the least vascular disease. A typical response in a normal subject is compared in Figure 5 with that in a patient with thromboangiitis obliterans. No noticeable change in pulse contour followed release of

occlusion in the one subject with thromboangiitis obliterans in whom the brachial intraarterial pressure was recorded (Figure 6). Furthermore, although a pressure drop occurred, it was less abrupt than in normals. The data are shown in Table V and the results of a typical experiment in Figure 7.

6. *Circulatory changes upon application of arterial and venous occlusion.* The changes in heart rate on application of arterial occlusion were not as consistent as those following release of occlusion. In 40 instances, application of bilateral arterial occlusion resulted in no significant change in

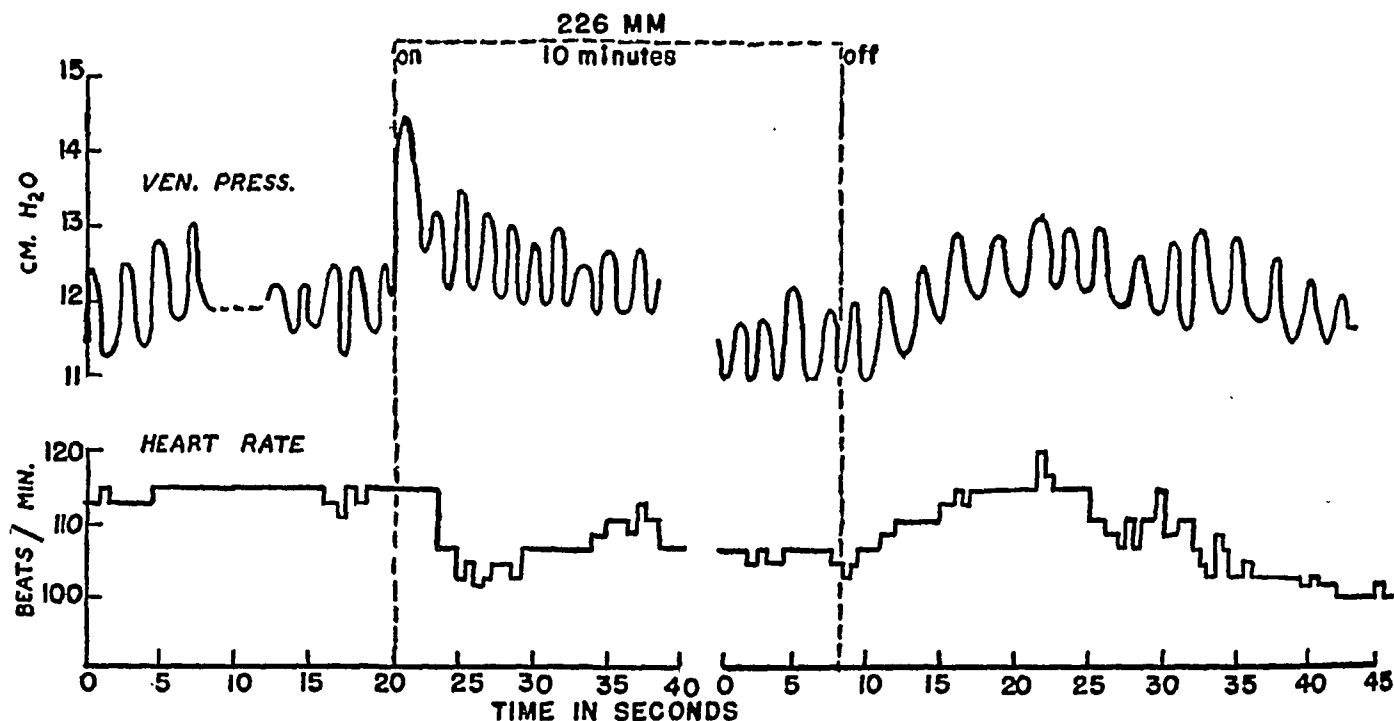


FIG. 4. A TYPICAL RESPONSE OF FEMORAL VEIN PRESSURE ON APPLICATION OF, DURING, AND FOLLOWING RELEASE OF BILATERAL ARTERIAL OCCLUSION OF THE LOWER EXTREMITIES IN A NORMAL SUBJECT

Chart constructed from optically recorded venous pressure, with a needle in the femoral vein. Heart rate was recorded with the electrocardiograph at camera speed of 2.5 cm. per second. Note the respiratory undulations in venous pressure.

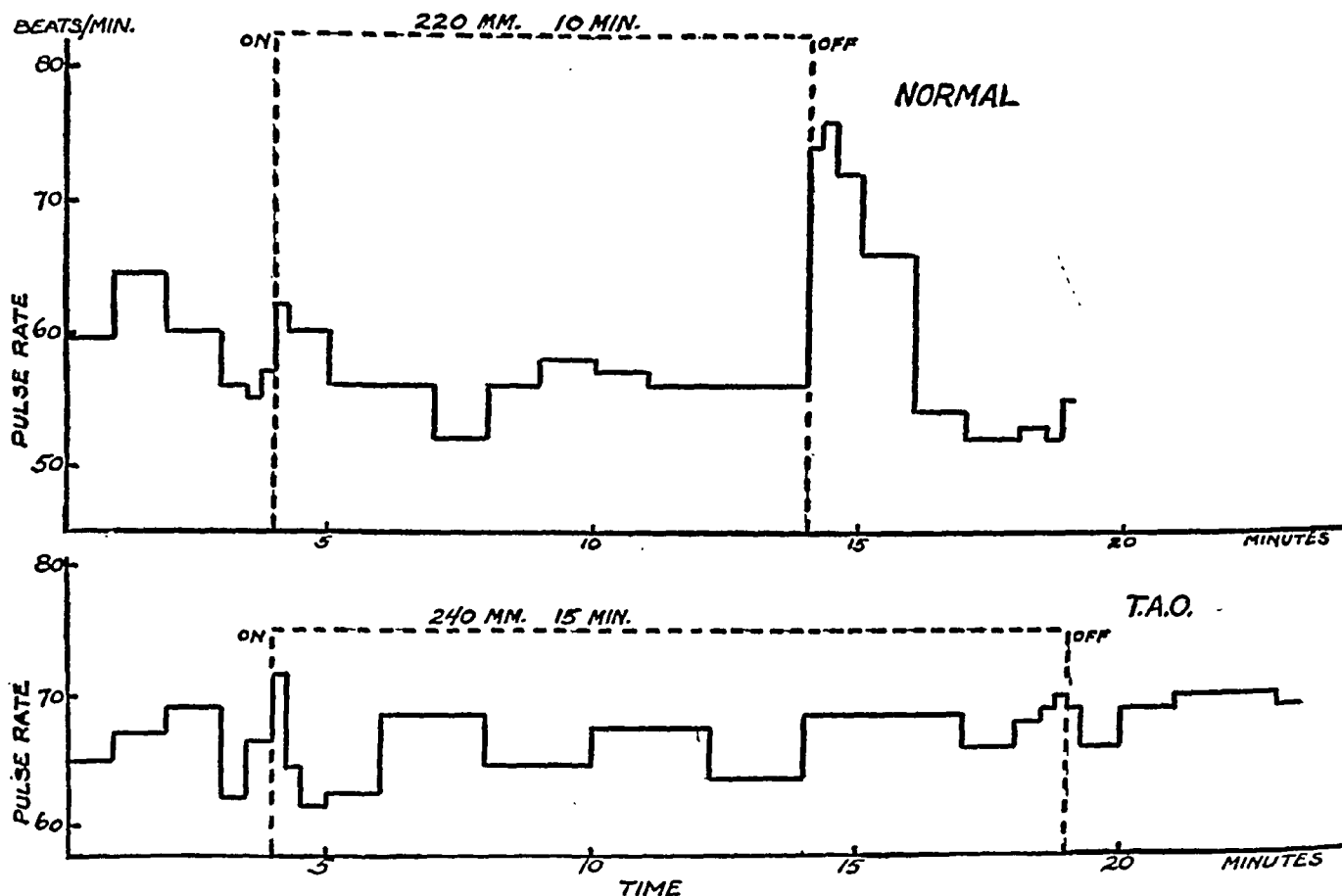


FIG. 5. A COMPARISON OF THE HEART RATE CHANGES IN A NORMAL SUBJECT (ABOVE) AND A PATIENT WITH THROMBOANGITIS OBLITERANS (BELOW) FOLLOWING RELEASE OF BILATERAL ARTERIAL OCCLUSION OF THE LOWER EXTREMITIES

Heart rate was recorded with the electrocardiograph at camera speed of 6 mm. per second.

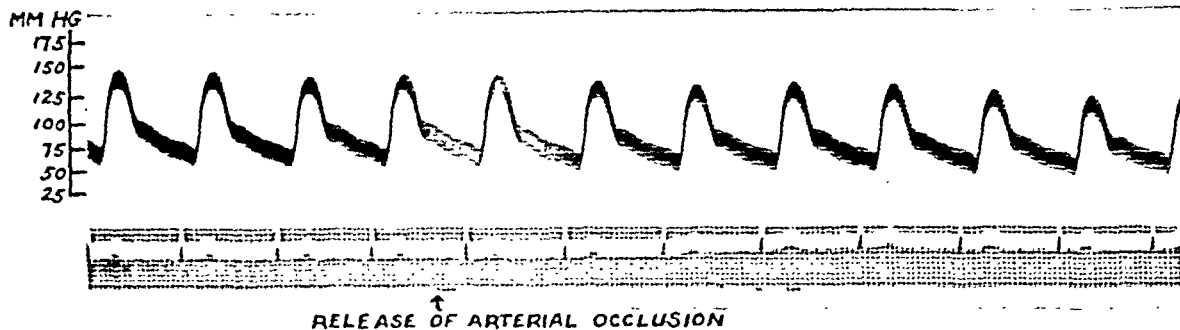


FIG. 6. A RECORD OF THE CHANGES IN BLOOD PRESSURE ON RELEASE OF BILATERAL ARTERIAL OCCLUSION OF THE LOWER EXTREMITIES IN A PATIENT WITH THROMBOANGIITIS OBLITERANS

The blood pressure was optically recorded with the Hamilton needle manometer in the brachial artery. Thin time lines on electrocardiogram equal 0.04 second and heavy lines equal 0.2 second. The arrow indicates the instant of release of occlusion, recorded as a dip by the signal magnet.

TABLE V

Brachial intraarterial pressure changes on release of arterial occlusion in one subject with thromboangiitis obliterans

Subject	Duration of occlusion	Maximum resting variation*	Maximum fall in systolic pressure	Maximum fall in diastolic pressure	Maximum fall in level of dirotic notch	Time of onset of blood pressure drop	Duration of blood pressure drop
	min-utes	mm. Hg	mm. Hg	mm. Hg	mm. Hg	seconds	seconds
MA	15	10	17	5	11	0.8	>45
EK	10	5	22	14	18	0.8	20
MA	3	9	12	7	8	Instantaneous	18.5

* Systolic and diastolic.

heart rate; while in 32 instances the heart rate slowed (Figure 4); and in 7 there was a pulse acceleration (*cf.* normal in Figure 5). In a number of instances, the acceleration was followed by a distinct slowing to below resting levels. Training failed to produce consistency in these changes.

When the limbs were previously made hyperemic by heat, occlusion caused a more consistent and a greater pulse slowing. Similarly, consistent slowing occurred when occlusion was applied during a period of reactive hyperemia. Slowing was greatest in this last circumstance.

More regular slowing followed application of venous occlusion, with slowing in 7 out of 10 trials.

In contrast to the heart rate, the arterial and venous pressures showed consistent changes on cuff inflation. In 5 observations on three normal subjects, a sharp and immediate rise in blood pressure accompanied inflation of the cuffs (Figure 1).

The pulse contour changed in a manner consistent with increased peripheral resistance, *i.e.*, the dirotic notch occurred higher on the curve (Figure 2). A summary of the data on arterial blood pressure is shown in Table VI.

TABLE VI

Blood pressure rise with application of occluding cuffs

Subject	Maximum resting variation*	Maximum rise in systolic pressure	Maximum rise in diastolic pressure	Maximum rise in level of dirotic notch	Duration of rise	
	mm. Hg	mm. Hg	mm. Hg	mm. Hg	Beats	Seconds
EK	4	10	21	24	3	2.7
EK	7	5	14	8	3	2.7
EK	8	5	6	12	3	3
KAM	5	15	16	20	>6	?
G	7	9	6	5	5	3.7

* Systolic and diastolic.

The venous pressure in the femoral vein proximal to the cuff fell during the period of occlusion (Table III). A typical curve is shown in Figure 4. At the instant of cuff inflation a momentary rise in femoral vein pressure occurred, which was apparently caused by the impact of the occluding cuff.

That some redistribution of blood had occurred is shown by the fact that in one normal subject, in each of 3 tests, the venous pressure in the antecubital vein rose during the period of lower limb occlusion only to drop again when the occlusion was released.

In the four patients with thromboangiitis obliterans only 2 out of 10 tests resulted in slowing

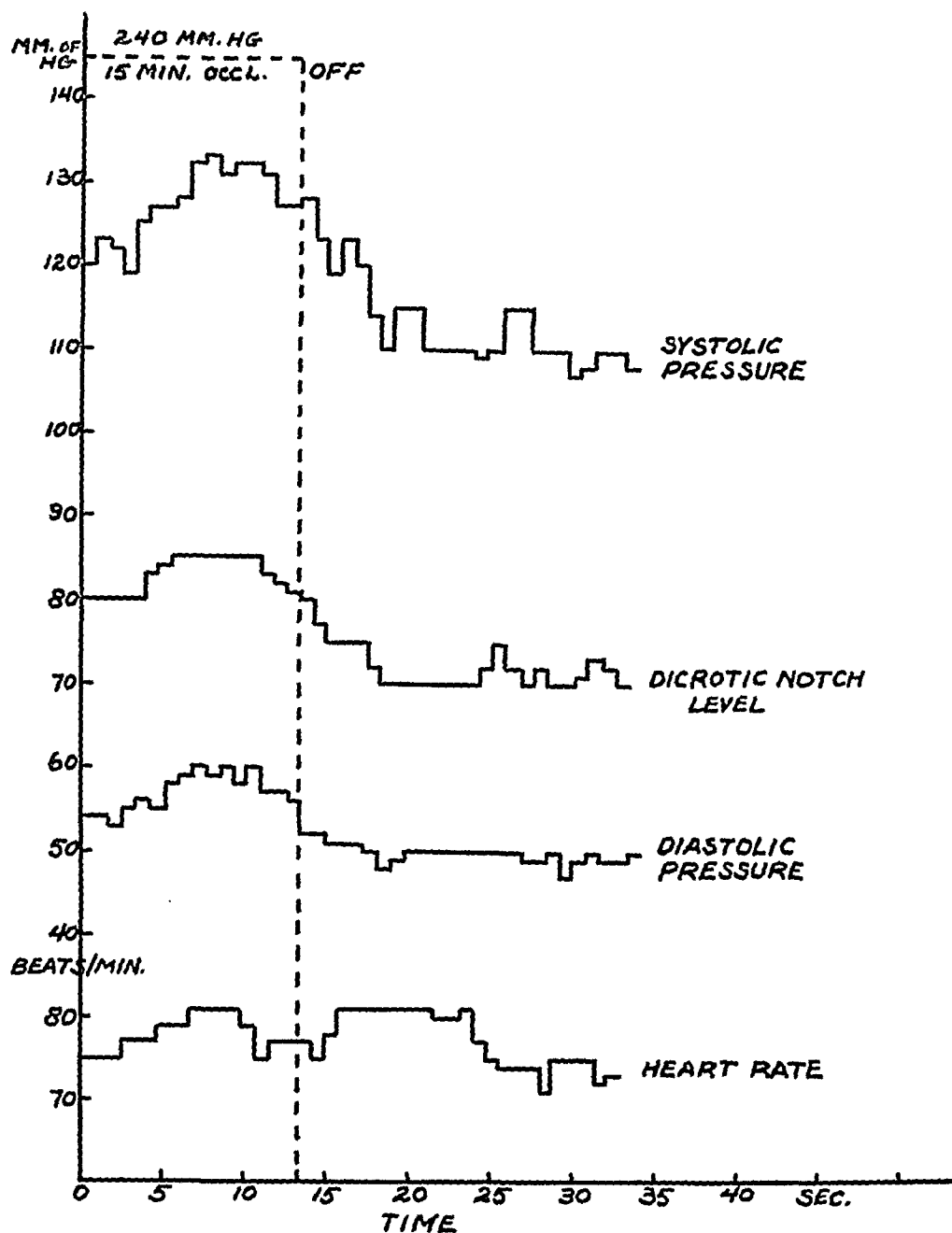


FIG. 7. THE EFFECT ON BLOOD PRESSURE AND HEART RATE OF RELEASE OF BILATERAL ARTERIAL OCCLUSION OF THE LOWER EXTREMITIES IN A PATIENT WITH THROMBOANGIITIS OBLITERANS

The blood pressure was recorded with the Hamilton needle manometer in the brachial artery and the heart rate with the electrocardiograph at camera speed of 2.5 cm. per second. Note that the fall in pressure on release is more gradual than in the normal subject (*cf.* Figure 2) and that no significant rise in heart rate occurs.

of the pulse on application of arterial occlusion. Figure 5 shows a negative experiment.

DISCUSSION AND INTERPRETATION OF RESULTS

Cardiac acceleration uniformly followed release of arterial occluding cuffs in normal subjects. This was preceded by a fall in brachial blood pressure (Figure 1). The fall in blood pressure and the change in pulse contour (Figure 2) demon-

strate the presence of a low-resistance path or shunt opened up on release of the constricting cuffs. When tissues are acutely deprived of blood, vascular dilatation occurs (19). There is therefore no need to suppose "pressor-sensitive zones" in the limb vessels initiating a depressor reflex, as postulated by Asmussen *et al.* (3 to 9). The pressure drop is apparently the result of the passage of blood into and through the dilated ves-

sels, the effect of this created path of low resistance being similar to that observed on releasing an A-V aneurysm (11, 18, 20). Lewis and Grant (19) have shown that the longer the period of vascular occlusion, the more marked and persistent is the reactive hyperemia on release. Table II shows the greater fall in pressure noted upon release of the more prolonged occlusions, which is consistent with this relationship. The fact that the cardiac acceleration (Figure 3), like the blood pressure drop, varies with the duration of prior occlusion, reinforces the hypothesis that the local vascular dilatation of reactive hyperemia is the initiating mechanism for both.

The time interval between the fall in blood pressure and the onset of cardiac acceleration (Table II) would permit a reflex acceleration to be initiated. This might arise in the end organs of the buffer nerves of the large vessels (*viz.*, root of aorta and carotid sinus) or from end organs in vessels or tissues of the limbs themselves. The time interval is, however, too short to permit the possibility that metabolites carried in the blood from the limbs might stimulate end organs in the arterial tree, the pacemaker of the heart, or central nervous system cardioregulatory centers. These mechanisms can thus be excluded. In like manner, a Bainbridge or allied reflex appears to be excluded.

Since the venous pressure rise occurred after the pulse acceleration had begun (Figure 4), it cannot be the initiating mechanism for the pulse acceleration. On similar ground, neither can an increased venous return to the heart be responsible. In line with this conclusion is the fact that venous pressure changes of similar magnitude occur during respiration without any significant change in heart rate. The possibility cannot, however, be excluded that the venous pressure rise and increased venous return contribute to maintaining the pulse acceleration. There is also the possibility that a volume increase might appear in the veins earlier than a detectable rise in pressure and cause the pulse acceleration, but this seems unlikely.

Results in two subjects with incomplete release of occlusion further support the view that the sudden opening of a by-path into which blood can flow will drop the systemic arterial pressure and so bring about the accelerator reflex. It was found that deflation to above diastolic pressure levels

failed to evoke cardiac acceleration, but deflation to below diastolic pressure levels resulted in an acceleration. In both cases pressure changes were transmitted to the distal limb, but in the former case no appreciable blood inflow occurred, and no acceleration was observed. This is evidence against the activity of local "pressor-sensitive zones." In the latter instance, when deflation to lower-than-diastolic pressure resulted in cardiac acceleration, congestion of the limb was produced. This is evidence indicating that an augmentation of venous return to the heart is not essential for the pulse acceleration.

It has been shown by Lewis and Grant (19) that venous occlusion, like arterial, will cause reactive hyperemia, but of lesser degree. In keeping with this, cardiac acceleration was observed to follow release of venous occlusion in 13 experiments on four subjects, but this was less in degree than that following arterial occlusion. However, the magnitude was masked by the discomfort of limb congestion, which in itself resulted in an acceleration during occlusion, except in those subjects who were exceptionally well trained.

The fact that no significant acceleration was observed in subjects who had thromboangiitis obliterans indicates again that the primary factor in the production of cardiac acceleration is the presence of a blood pathway of low resistance. In these subjects, whether or not dilatation is present or is produced by a period of arterial occlusion, the compromised arterial lumen is a fixed factor limiting the blood inflow (17), maintaining a high total resistance, and preventing the augmentation of inflow normally observed to follow dilating procedures (14, 16).

When the occluding cuffs were inflated, the results were not exactly the reverse of those observed upon release of occlusion. On application of occlusion, the situation differs from that encountered in compressing an A-V aneurysm. Since occlusions were usually applied during resting states, the effect was not that of blocking an augmented circulation through a dilated system or shunt. Further, some tendency to cardiac acceleration may be ascribed to the sensation of cuff inflation. When dilation or a by-path was present at the time of application of occlusion, induced either by heat or by re-occluding during a period of reactive hyperemia, the cardiac slowing was

apparent. The change in arterial pressure reflected in direction and in contour (Figure 2) an increase in peripheral resistance. Consistent, too, is the observation that the subjects with thromboangiitis obliterans had a reduced or absent response to this manoeuvre.

CONCLUSIONS

1. The phenomena associated with the application and release of constricting tourniquets were observed.

2. Cardiac acceleration followed release of occluding cuffs about the lower extremities in normal subjects. This was absent or reduced in patients with thromboangiitis obliterans.

3. A fall in the blood pressure in the brachial artery preceded the cardiac acceleration.

4. The fall in blood pressure was caused by the opening of a temporary low resistance pathway for blood through the dilated vessels resulting from the previous occlusion of the limbs.

5. Evidence is cited to prove that the primary mechanism inducing the cardiac acceleration is a reflex response to the drop in pressure in the central arteries (Marey's Law).

6. The evidence presented also indicates that the cardiac acceleration is not caused by a metabolite accumulating in the constricted extremities; that it is not satisfied by the assumption that the reflex arises from the occluded vessel or from the tissue of the extremity.

BIBLIOGRAPHY

1. Alam, M., and Smirk, F. H., Observations in man upon a blood pressure raising reflex arising from the voluntary muscles. *J. Physiol.*, 1937, 89, 372.
2. Alam, M., and Smirk, F. H., Observations in man on a pulse-accelerator reflex from the voluntary muscles of the legs. *J. Physiol.*, 1938, 92, 167.
3. Asmussen, E., Christensen, E. H., and Nielsen, M., On the influence on pulse rate of alterations of arterial blood pressure. *Skandinav. Arch. f. Physiol.*, 1938, 79, 32.
4. *Idem*, Influence of blood distribution on circulation and physical exercise. *Ibid.*, 1939, 81, 185.
5. *Idem*, Posture and pulse rate. *Ibid.*, 1939, 81, 190.
6. *Idem*, The effect on blood pressure regulation of various postures. *Ibid.*, 1939, 81, 204.
7. *Idem*, On the circulatory insufficiency in the standing posture with normal arterial pressure and reduced minute-volume. *Ibid.*, 1939, 81, 214.
8. *Idem*, The significance of posture on pulse rate with exercise. *Ibid.*, 1939, 81, 224.
9. *Idem*, The regulation of the circulation in different postures. *Surgery*, 1940, 8, 604.
10. Hamilton, W. F., Brewer, J., and Brotman, I., Pressure pulse contours in the intact animal. *Am. J. Physiol.*, 1934, 107, 427.
11. Holman, E., The anatomic and physiologic effects of an arteriovenous fistula. *Surgery*, 1940, 8, 362.
12. Jarisch and Gaisböck, Concerning the state of the circulation in reactive hyperemia. *Arch. f. exper. Path. u. Pharmacol.*, 1929, 139, 159.
13. Krogh, A., Effect of posture on the regulation of the circulation. *Proc. Inst. Med. Chicago*, 1939, 12, 398.
14. Kunkel, P., and Stead, E. A., Jr., Blood flow and vasomotor reactions in the foot in health, in arteriosclerosis and in thromboangiitis obliterans. *J. Clin. Invest.*, 1938, 17, 715.
15. Landowne, M., Simple apparatus for optical registration of vascular dynamics. *Am. J. Physiol.*, 1941, 133, 359.
16. Landowne, M., and Katz, L. N., A critique of the plethysmographic method of measuring blood flow in the extremities of man. *Am. Heart J.* (In press.)
17. Landowne, M., Dynamics of blood flow in thromboangiitis obliterans. (Unpublished.)
18. Lewis, D., Libman Anniversary Volume. The bradycardiac reaction and the cardiac changes in arteriovenous aneurisms. International Press, New York. 1932, II, 733.
19. Lewis, T., and Grant, R., Observations upon reactive hyperemia in man. *Heart*, 1925, 12, 73.
20. McGuire, J., Hauenstein, V., Stevens, C. D., and Sharrets, K. C., Effects of arteriovenous fistulae on the heart and circulation. Blood, Heart and Circulation, Publication No. 13 of Amer. Assoc. Advancement Sci., 1940, p. 213.

THE FILTRATION RATE, EFFECTIVE RENAL BLOOD FLOW, TUBULAR EXCRETORY MASS AND PHENOL RED CLEARANCE IN NORMAL PREGNANCY¹

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Alterations in kidney physiology have long been sought to explain certain phenomena of normal pregnancy. Most suggestive of a renal disturbance was the well known tendency of pregnant women to retain salt and water, clinical evidence of which is found in the edema of late pregnancy and in the diuresis of the first few days postpartum. There was also the apparent readiness with which pregnant women developed specific toxemia with its proteinuria. It had indeed been suggested that certain types of toxemia were caused by the alleged strain of pregnancy imposed on the "low reserve" kidney (1).

Additional interest has been given this subject by recent observations that the steroid hormones of the testis and the ovary cause an increased retention of salt and water (2, 3, 4, 5) and when injected into rats and mice lead to a considerable increase in the size of the kidney (6, 7, 8). Two of these substances, estrogen and progesterone, are produced by the placenta and are present in great concentration in the body fluids of all pregnant women. Good reasons therefore exist for considering the possibility of change in kidney physiology, although no consistent abnormality in renal function has yet been demonstrated in normal pregnancy (9, 10, 11).

The present report concerns the measurements of glomerular filtration, effective renal blood flow and the total functioning tubular tissue in normal pregnant women. Tests were carried out during pregnancy and were repeated after delivery, so that the effects of delivery on the kidney function of a single individual can be studied.

The rate of filtration at the glomerulus is determined by the inulin clearance, the rate of effective renal blood flow by the diodrast clearance, and the

maximum tubular excretory function or tubular excretory mass by diodrast T_m . The physiological basis for these measurements has been reviewed by Smith and his associates (12, 13, 14, 15). Smith has also recently discussed the functional significance and limitations of renal clearances (16).

MATERIAL

For the tests patients were selected from the prenatal clinic or the ward. Fifteen women were studied in the last lunar month, three in the ninth month, and three in early pregnancy. Nine of these twenty-one were again tested after delivery. None had hypertension, proteinuria, edema, or a history of kidney disease, hypertension or specific toxemia of pregnancy.

METHOD

The patient was given a liter of water to drink the evening before the test. The next morning between 7:30 and 8:30 she was given another liter. No breakfast was allowed.

Inulin, phenol red and diodrast were given by continuous intravenous infusion at a controlled rate. When this work was begun in May, 1938, physiological saline was used in the infusion fluid. At the same time, tests were started on patients with specific toxemia of pregnancy in whom it was soon found that an adequate urine flow could not be obtained by this method. A two per cent solution of sodium sulphate (anhydrous) in saline was tried next, and later an eight per cent solution of sorbitol or mannitol in distilled water was used.² From July, 1939 to the present time eight per cent mannitol in distilled water has been employed routinely. With this infusion fluid, the urine flows have ranged from two to ten cc. per minute, usually being about five cc. per minute. The error in clearance values resulting from pos-

² We are indebted to the Winthrop Chemical Company for the diodrast and to the Abbott Laboratories for the mannitol used in this investigation. The inulin used was prepared by the U. S. Standard Products Company, the phenol red by Messrs. Hynson, Westcott and Dunning, and the Sterisol saline and distilled water by Schering and Glatz, Inc.

¹ This study was made with the aid of a grant from the Commonwealth Fund.

sible incomplete emptying of the bladder was minimized by maintaining a high urine flow. Any test in which this was below two cc. per minute was discarded.

Urine specimens were collected by a six-holed rubber catheter left in the bladder throughout the test. At the end of each urine collection period the bladder was washed out with physiological saline followed immediately by insufflation with air. Care in this step is particularly necessary in studying patients in late pregnancy, because the fetal head makes impossible the suprapubic pressure usually employed to insure complete emptying.

Two or three venous blood samples were taken during the clearance periods, and three during the diodrast T_m periods. Plasma levels of diodrast, phenol red and inulin were interpolated to the midpoint of each urine collection period.

Blood pressures, pulse rates, and mouth temperatures were taken at frequent intervals throughout the test. Hematocrits were determined on the sample of blood taken just before the infusion was begun. The first urine sample was examined routinely for protein and formed elements.

Iodine was analyzed by the Kendall method (17). Inulin was analyzed by the method of Folin (18, 19) in the earlier experiments and by that of Alving, Rubin and Miller (20) in the more recent ones. Certain modifications of these methods were introduced according to published directions by Smith *et al.* and Goldring *et al.* (14, 21).

RESULTS

Inulin and phenol red clearances were done on twenty normal pregnant women, with diodrast clearance in eleven and diodrast T_m in eight. After delivery inulin and phenol red clearances were done on ten women, with diodrast clearance and diodrast T_m in six.

The values for the three clearances, the diodrast T_m and the significant ratios are given in Table I. Each clearance figure is the average of three or more urine collection periods, each diodrast T_m the average of five. The inulin clearances average 124 cc. per minute antepartum and 116 cc. per minute postpartum; the phenol red clearances average 371 cc. per minute antepartum and 362 cc. per minute postpartum; diodrast clearances average 631 cc. per minute antepartum and 525 cc. per minute postpartum. The effective renal blood flow appears in the table only in the cases in which diodrast clearances were done. Before delivery the average value is 970 cc. per minute and after delivery it is 858 cc. per minute.

The filtration fraction, or inulin/diodrast clearance ratio, which measures the proportion of the effective plasma flow filtered at the glomerulus,

is nearly identical antepartum and postpartum. The inulin/phenol red clearance ratio is used to indicate changes in filtration fraction in those instances where diodrast clearances were not done, on the assumption that any change in inulin/phenol red clearance ratio reflects a similar change in filtration fraction of the same functional significance, if not of identical degree. The figures for this ratio antepartum and postpartum are likewise nearly identical. The phenol red/diodrast clearance ratio, although varying widely in different individuals, shows no consistent change between antepartum and postpartum observations in the same individuals.

The diodrast T_m averages 45.6 mgm. of iodine per minute antepartum and 46.6 mgm. of iodine per minute in postpartum observations. In the last three columns the effective renal blood flow, diodrast clearance, and inulin clearance have been related to the diodrast T_m . This is a convenient way in which to compare figures in different individuals because kidney function as measured by the respective clearances is related to standard amounts of renal tissue and variations due to kidney size are thereby eliminated.

Review of the data in Table I shows that no significant changes occur in inulin clearance, phenol red clearance, diodrast clearance or diodrast T_m in pregnancy when renal function is compared to the postpartum observations as a standard of reference.

Comparison of the average figures from the smaller groups made up only from those individuals on whom both antepartum and postpartum observations were made (Table II) again shows no significant differences. Diodrast clearance and diodrast T_m were determined in only three subjects both antepartum and postpartum. While this is a small group to average, the figures again indicate no change in renal function.

Comparison of our results in pregnancy and the puerperium with those in normal non-pregnant women, as observed by Goldring, Chasis, Ranges and Smith (21), and normal non-pregnant and pregnant women, as observed by Chesley and Chesley (10, 11) appears in Table III. Our clearances, ratios and clearances per unit T_m in pregnancy are almost identical with those in non-pregnant women, as reported by Goldring *et al.*

TABLE I
Results of kidney function tests in normal pregnancy and in the puerperium

Subject	Infusion fluid	Sur- face area	Date	Duration of preg- nancy in weeks by history	Time in weeks before delivery	Mean blood pressure during test	Antepartum					Effective renal blood flow/ $T_m D$	Phenol red/ Diodrast	Diodrast T_m mgm. iodine per minute	Effective renal blood flow/ $T_m D$	$C_{IN}/$ $T_m D$	$C_D/$ $T_m D$	
							Plasma clearances		Effective renal blood flow cc. per 1.73 sq.m. per minute	Inulin/ Phenol red	Filtration fraction Inulin/ Diodrast							
							Inulin	Phenol red										
1. D. R.	(a)	sq.m.	May 26, 1938	41	1	128/70	140	404		712	31.7							
2. L. P.	(b)	1.51	July 7, 1938	40	0	124/50	93	290		1220	30.7	16.8			18.1*	11.8*		
3. C. B.	(b)	1.58	August 11, 1938	40	1	128/88	91	276		983	32.6	18.6			17.3	11.1	2.02	
4. S. M.	(b)	1.50	August 18, 1938	41	2	120/80	169	471		845	33.8	27.5			20.1	11.2	3.02	
5. H. D.	(b)	1.65	September 22, 1938	41	1	122/80	128	382		833	33.5	21.3			25.1	15.8	3.32	
6. H. Q.	(b)	1.38	October 13, 1938	38	1	120/80	135	403		774	32.6	20.9			23.8	16.6	2.36	
7. R. S.	(b)	1.37	October 19, 1938	39	2	110/80	138	430		1241	32.6	15.4			15.7	10.2†	2.49	
8. W. O.	(a)	1.58	November 9, 1938	40	1	120/80	86	293		518	33.5	21.5			22.7	11.5	3.02	
9. B. D.	(a)	1.48	January 5, 1939	40	1	110/70	114	281		1010	31.6	20.9						
10. E. S.	(a)	1.83	October 28, 1940	40	2	110/70	116	297		462	30.7	16.8						
11. V. M.	(c)	1.58	October 18, 1940	40	1	112/72	134	321		797	32.6	18.6						
12. G. R.	(c)	1.65	September 12, 1940	40	1	110/68	123	378		662	32.6	18.6						
13. M. B.	(c)	1.55	December 16, 1940	38	4	118/70	99	360		545	32.6	18.6						
14. M. E.	(c)	1.66	May 31, 1939	32	8	120/76	125	370		586	33.5	21.3						
15. A. B.	(c)	1.60	May 11, 1939	34	4	116/78	125	370		774	32.6	20.9						
16. L. D.	(c)	1.37	June 22, 1939	14	24	120/76	162	428		800	32.6	15.4						
17. H. H.	(c)	1.50	December 6, 1939	12	7	134/80	123	378		1241	32.6	15.4						
18. L. B.	(c)	1.75	January 18, 1940	40	0	108/78	134	400		518	33.5	21.5						
19. M. A.	(c)	1.55	November 27, 1939	13	27	105/66	139	402		665	31.6	20.9						
20. I. J.	(c)	1.68	February 27, 1940	26	14	124/80	138	474		616	29.1	21.4						
21. M. B. O.	(c)	1.53	April 27, 1940	34	3	124/80	85	260		457	32.7	18.6						
22. R. D.	(b)		May 1, 1940	40	1		124	371		631	33.4	19.6						
Averages.....										970				45.6	20.9	13.7	2.67	
Subject	Infusion fluid	Sur- face area	Date	Time in weeks after delivery	Mean blood pressure during test	Postpartum					Effective renal blood flow	Inulin/ Phenol red	Filtration fraction Inulin/ Diodrast	Phenol red/ Diodrast	Diodrast T_m mgm. iodine per minute	Effective renal blood flow/ $T_m D$	$C_{IN}/$ $T_m D$	$C_D/$ $T_m D$
						Plasma clearances		Effective renal blood flow cc. per 1.73 sq.m. per minute	Inulin/ Phenol red	Filtration fraction Inulin/ Diodrast								
						Inulin	Phenol red											
1. D. R.	(a)	sq.m.	(a) June 9, 1938	2	104/72	139	475		922	29.3								
2. L. P.	(b)	1.51																
3. C. B.	(b)	1.58																
4. S. M.	(b)	1.50																
5. H. D.	(b)	1.65																
6. H. Q.	(b)	1.38																
7. R. S.	(b)	1.37																
8. W. O.	(a)	1.58																
9. B. D.	(a)	1.48																
10. E. S.	(a)	1.83																
11. V. M.	(c)	1.55																
12. G. R.	(c)	1.65																
13. M. B.	(c)	1.55																
14. M. E.	(c)	1.66																
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17. H. H.	(c)	1.50																
18. L. B.	(c)	1.75																
19. M. A.	(c)	1.55																
20. I. J.	(c)	1.68																
21. M. B. O.	(c)	1.53																
22. R. D.	(b)																	
Averages.....										858	32.0	20.8		46.6	18.65	13.25	2.28	

(a) Infusion in saline and sodium sulphate. (c) Infusion in mannitol and distilled water. * Postpartum T_m used. † Average of two tests. ‡ In this patient, 18 L. B., $C_D/T_m D$ is abnormally low. There is, however, no clinical evidence or history of hypertension or kidney disease to warrant her exclusion from the group.

(a) Infusion in saline. (b) Infusion in saline and sodium sulphate. (c) Infusion in mannitol and distilled water. * Postpartum T_m used. † Average of two tests. ‡ In this patient, 18 L. B., $C_D/T_m D$ is abnormally low. There is, however, no clinical evidence or history of hypertension or kidney disease to warrant her exclusion from the group.

TABLE II
Averages of antepartum and postpartum observations in same patients

Number and type of case	Plasma clearances			Effective renal blood flow	Inulin/Phenol red	Inulin/Diodrast	Phenol red/Diodrast	Diodrast T_m	Effective renal blood flow/ T_{mD}	C_D/T_{mD}	C_{IN}/T_{mD}
	Inulin	Phenol red	Diodrast								
	cc. per 1.73 sq. m. per minute			cc. per 1.73 sq. m. per minute	per cent	per cent	per cent	mgm. iodine per minute			
9 antepartum	120	355			33.8						
9 postpartum	114	360			31.7						
3 antepartum	119	378	550	852	31.5	21.6	68.7	43.9	19.4	12.5	2.71
3 postpartum	105	331	486	800	31.7	21.6	68.1	44.9	17.8	10.8	2.34

TABLE III
Averages of normal pregnant women compared with normal non-pregnant women and normal men

Number and type of case	Plasma clearances			Effective renal blood flow	Inulin/Phenol red	Filtration fraction	Phenol red/Diodrast	Diodrast T_m	Effective renal blood flow/ T_{mD}	C_D/T_{mD}	C_{IN}/T_{mD}
	Inulin	Phenol red	Diodrast			Inulin/Diodrast					
	cc. per 1.73 sq. m. per minute			cc. per 1.73 sq. m. per minute	per cent	per cent	per cent	mgm. iodine per minute			
9 normal non-pregnant women*	119		600	996		19.8		46.7	21.3	12.8	2.54
30 normal men*					32.2						
15 normal non-pregnant women†			545	844							
15 normal pregnant women†			591	856							
20 normal pregnant women	124	371			33.4						
8 normal pregnant women	126	384	628	970	32.9	20.1	61.2	45.6	21.3	13.7	2.77

* Goldring, Chasis, Ranges and Smith.

† Chesley and Chesley.

The diodrast clearances are also essentially the same as those reported by Chesley and Chesley.³

Renal function as revealed by these tests is unaltered by normal pregnancy and undergoes no

³ Chesley's infusions contained only glucose and diodrast; infusions used by Goldring, Chasis, Ranges and Smith contained inulin and diodrast in saline; our infusions were made up of inulin, phenol red, diodrast and, in various instances, saline, sodium sulphate or sorbitol or mannitol. The close comparison of diodrast clearance figures found in these three groups seems to rule out Chesley's suggestion (10) that infusions of inulin and phenol red may cause a renal hyperemia.

change in the days immediately following delivery. One cannot therefore explain the slight salt and water retention of normal pregnancy on the basis of a decreased filtration rate. There is no evidence of any hormonal effect on kidney function which might accompany the morphological change known to follow the injection of similar substances into small laboratory animals.

CONCLUSION

The filtration rate (inulin clearance), effective renal blood flow (diodrast clearance), tubular ex-

cretory mass (diodrast T_m) and phenol red clearances are not altered in pregnancy or in the puerperium of normal women.

BIBLIOGRAPHY

1. Stander, H. J., and Kuder, K., Low reserve kidney. *Am. J. Obst. and Gynec.*, 1938, 35, 1.
2. Taylor, H. C., Jr., Warner, R. C., and Welsh, C. A., The relationship of the estrogens and other placental hormones to sodium and potassium balance at the end of pregnancy and in the puerperium. *Am. J. Obst. and Gynec.*, 1939, 38, 5, 748.
3. Thorn, G. W., Engel, L. L., and Eisenberg, H., Effect of corticosterone and related compounds on renal excretion of electrolytes. *J. Exper. Med.*, 1938, 68, 161.
4. Thorn, G. W., and Engel, L. L., Effect of sex hormones on renal excretion of electrolytes. *J. Exper. Med.*, 1938, 68, 299.
5. Thorn, G. W., Nelson, K., and Thorn, D. W., Study of mechanism of edema associated with menstruation. *Endocrinology*, 1938, 22, 155.
6. Ludden, J. B., Krueger, E., and Wright, I. S., Effect of testosterone propionate, estradiol benzoate, and desoxycorticosterone acetate on the kidneys of adult rats. *Endocrinology*, 1941, 28, 619.
7. Selye, H., The effect of testosterone on the kidney. *J. Urol.*, 1939, 42, 637.
8. Selye, H., On the protective action of testosterone against the kidney-damaging effect of sublimate. *J. Pharm. and Exper. Therap.*, 1940, 68, 454.
9. Chesley, L. C., Renal function tests in the differentiation of Bright's disease from so-called specific toxemia of pregnancy. *Surg., Gynec. and Obst.*, 1938, 67, 481.
10. Chesley, L. C., and Chesley, E. R., The diodrast clearance and renal blood flow in normal pregnant and non-pregnant women. *Am. J. Physiol.*, 1939, 127, 731.
11. Chesley, L. C., and Chesley, E. R., Renal blood flow in women with hypertension and renal impairment. *J. Clin. Invest.*, 1940, 19, 475.
12. Chasis, H., Ranges, H. A., Goldring, W., and Smith, H. W., The control of renal blood flow and glomerular filtration rate in normal man. *J. Clin. Invest.*, 1938, 17, 683.
13. Smith, H. W., *The Physiology of the Kidney*. Oxford University Press, New York, 1937.
14. Smith, H. W., Goldring, W., and Chasis, H., The measurement of the tubular excretory mass, effective blood flow, and filtration rate in the normal kidney. *J. Clin. Invest.*, 1938, 17, 263.
15. Smith, H. W., Chasis, H., Goldring, W., and Ranges, H. A., Glomerular dynamics in the normal kidney. *J. Clin. Invest.*, 1940, 19, 751.
16. Smith, H. W., Note on the interpretation of clearance methods in the diseased kidney. *J. Clin. Invest.*, 1941, 20, 631.
17. Kendall, E. C., Determination of iodine in connection with studies in thyroid activity. Third paper. *J. Biol. Chem.*, 1920, 43, 149.
18. Folin, O., Two revised copper methods for blood sugar determinations. *J. Biol. Chem.*, 1929, 82, 83.
19. Samoggi, M., A method for the preparation of blood filtrates for the determination of sugar. *J. Biol. Chem.*, 1930, 86, 655.
20. Alving, A. S., Rubin, J., and Miller, B. F., A direct colorimetric method for the determination of inulin in blood and urine. *J. Biol. Chem.*, 1939, 127, 609.
21. Goldring, W., Chasis, H., Ranges, H. A., and Smith, H. W., Relations of effective renal blood flow and glomerular filtration to tubular excretory mass in normal man. *J. Clin. Invest.*, 1940, 19, 739.

THE FILTRATION RATE, EFFECTIVE RENAL BLOOD FLOW, TUBULAR EXCRETORY MASS AND PHENOL RED CLEARANCE IN SPECIFIC TOXEMIA OF PREGNANCY¹

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The vascular and renal disorders of the last trimester of pregnancy are classified by the University Obstetrical Service in the following groups: specific toxemia, pre-existing essential hypertension, pre-existing glomerulonephritis and specific toxemia superimposed on pre-existing hypertension or nephritis (1).

Specific toxemia is clinically characterized by the rather abrupt appearance of hypertension, usually in the last trimester of a previously normal pregnancy. This rise in blood pressure is typically accompanied by edema and proteinuria. Hematuria and azotemia are not seen, but the convulsions of eclampsia may occur. The postpartum course of the disease likewise helps to identify it. Edema subsides soon after delivery. Proteinuria usually disappears in the puerperium, although traces of protein in the urine may be present for weeks. Blood pressure falls to a normal level in the majority soon after delivery, but it is a striking feature of such an attack that in many instances it is followed by permanent hypertension.

The present communication is an investigation of renal function in specific toxemia of pregnancy, based upon a study of inulin, phenol red and diodrast clearances and diodrast T_m . The inulin clearance is considered to be a measure of the rate of glomerular filtration, diodrast clearance a measure of effective renal blood flow and diodrast T_m a measure of tubular excretory mass (2, 3), within the definitions and limitations accorded to these terms by Smith (4).

Using these methods, Goldring *et al.* (5, 6, 7, 8) have demonstrated that renal ischemia of varying degree, associated with an increase in filtration fraction which is indicative of efferent arteriolar

hypertonus, is usually present in essential hypertension in men and non-pregnant women. Chesley *et al.* (9) have shown that the effective renal blood flow in toxemia of pregnancy, as determined by the diodrast clearance of twenty patients, is the same as that of normal pregnant and non-pregnant women. Corcoran and Page (10), using clearances of inulin and phenol red, found the filtration rate diminished, effective renal blood flow normal and the phenol red/inulin clearance ratio high during toxemia in seven subjects when compared to their postpartum values. In three of these subjects, in whom diodrast clearance was determined both antepartum and postpartum, they observed a fall in diodrast clearance and a rise in filtration fraction after delivery. They attribute the antepartum decrease in filtration fraction to swelling of the glomerular basement membrane. That the glomerular filtration rate and effective renal blood flow are unaffected in normal pregnancy was concluded in our previous report (11). This was based on inulin and phenol red clearances in twenty normal pregnant women, with diodrast clearance in eleven and diodrast T_m in eight.

MATERIAL AND METHODS

Thirteen patients, who had been seen early in pregnancy and found at that time to have no evidence of vascular or renal disease, have been studied in fourteen pregnancies complicated by specific toxemia. None gave a history of previous hypertension or kidney disease except for one patient who, though her blood pressure was normal between gestations, had developed a typical attack of specific toxemia in a previous pregnancy. In the last trimester, hypertension, proteinuria and edema appeared in all of these patients but, as may be seen in Tables I and II, proteinuria and edema had disappeared in some instances before the day of the antepartum tests. Two patients developed the typical antepartum convulsions of eclampsia and there were three intrauterine fetal deaths.

¹ This study was made with the aid of a grant from the Commonwealth Fund.

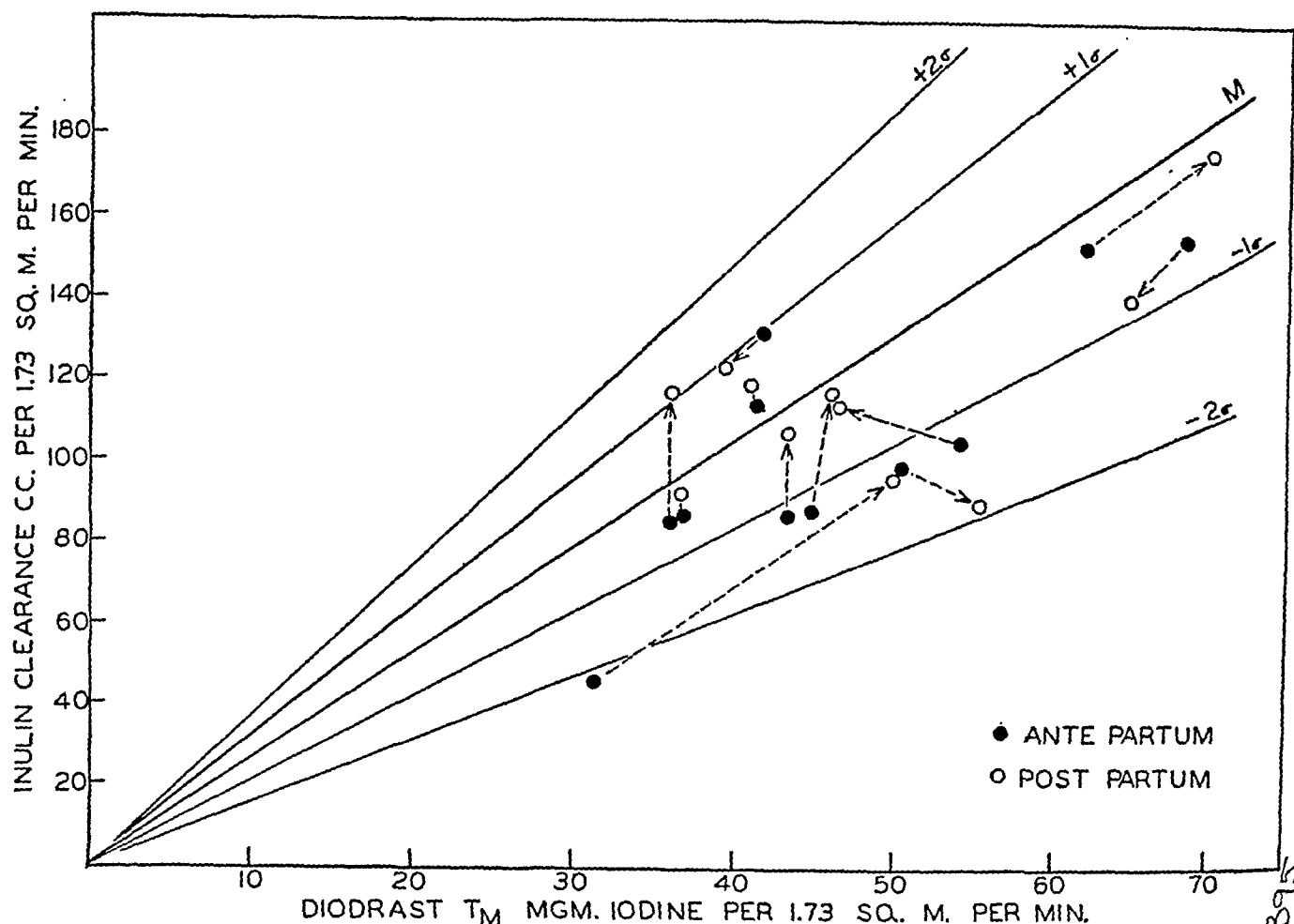


FIG. 1. COMPARISON OF ANTEPARTUM WITH POSTPARTUM INULIN CLEARANCES

ance per unit of diodrast T_m . The horizontal line at 0.20 represents the mean normal value for the filtration fraction. The vertical line at 13.2 is the mean normal value of diodrast clearance per unit of diodrast T_m . The central curved line is the course of the variables if the glomerular filtration rate maintains its mean normal value at varying rates of renal plasma flow. The parallel curved lines are $+2\sigma$ and -2σ .

The figures for filtration fraction before delivery are below the lowest normal value in only five of eleven subjects. Yet it can be seen that all of the antepartum filtration fractions are on or below the normal line and that all of the arrows point upwards, showing the increase in filtration fraction after delivery. The antepartum reduction in filtration fraction is dependent upon an increase in diodrast clearance, a reduction in inulin clearance, or both. After delivery it rises without exception, in part because of an increased inulin clearance and in part because of a decreased diodrast clearance, the postpartum rise being greater in the group with persistent hypertension.

Phenol red clearance. The clearance of phenol red (Tables I and II), like that of diodrast, suffers a reduction after delivery, but to a lesser extent. It can be seen also that the inulin/phenol red clearance ratio is somewhat reduced antepartum and increased after delivery, a phenomenon not observed in normal pregnancy (11). Although the variations from the normal in this ratio are small, they follow the changes in filtration fraction in direction and time and may be considered to have the same functional significance.

Although the phenol red/diodrast clearance ratio varies over a wide range in antepartum and postpartum observations, it rises consistently after delivery in all instances where the diodrast clearance falls. This increase in ratio accompanying a decrease in diodrast clearance is in agreement with the observation of Chasis *et al.* that the phenol red/diodrast clearance ratio varies inversely as the renal blood flow (13). Calculation of renal blood flow from phenol red clearance in subjects with toxemia of pregnancy, especially

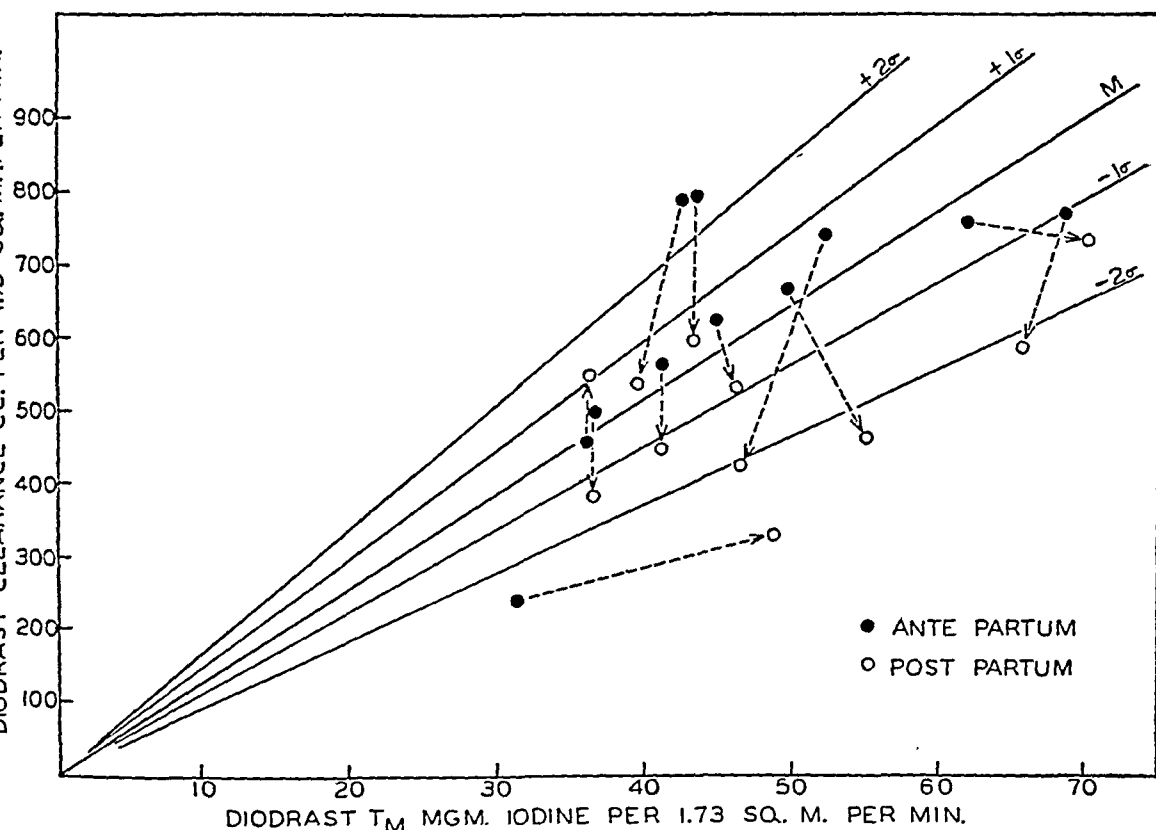


FIG. 2. COMPARISON OF ANTEPARTUM WITH POSTPARTUM DIODRAST CLEARANCES

For a comparison of antepartum with postpartum renal function, is evidently subject to greater error than is such calculation in normal subjects.

DISCUSSION

When renal function in fourteen pregnancies complicated by specific toxemia is compared to that of the control group, the clearances are found below the normal range in only two, while the filtration fraction is below normal in five. In other words, if judged from antepartum observations alone, one-half of this series of toxemic patients would appear to have normal kidney function. However, when these patients are considered as a group in comparison with the normals, as well as when antepartum figures are compared with postpartum figures, trends become evident which are not found in the pregnancy or puerperium of normal women. Before delivery there is a slight reduction in filtration rate and filtration fraction associated with a normal or slightly elevated effective renal blood flow. Fol-

lowing delivery the filtration fraction rises in every instance, in part because of a rise in filtration rate and in part because of a fall in effective renal blood flow. In the group with residual hypertension the increase in filtration fraction is more marked than in the group with clinical cure and reaches levels seen in essential hypertension (5, 6, 8).

The antepartum clearances and clearance ratios bear no consistent relation to the severity of the clinical manifestations or to the eventual outcome of the disease. The postpartum observations, however, fall readily into two groups and may offer some aid in prognosis. In the patients in whom hypertension was to persist, the diodrast clearance had fallen and the filtration fraction had risen considerably in the first test done postpartum, whereas in the cured group the inulin clearance had increased, the diodrast clearance had fallen slightly, and the filtration fraction had risen to normal in the first test done after delivery, regardless of whether the blood pressure had fallen to normal or not.

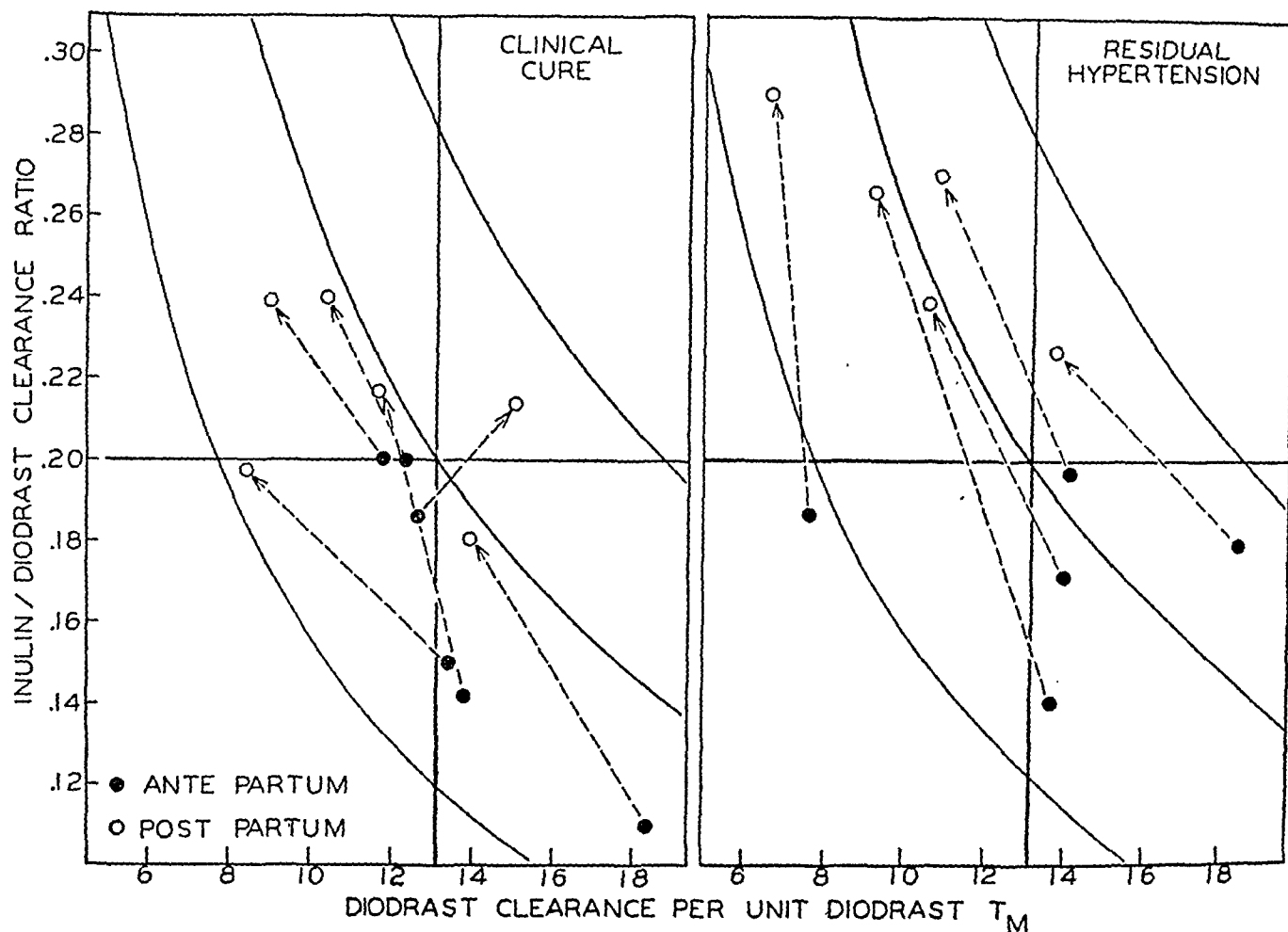


FIG. 3. COMPARISON OF ANTEPARTUM WITH POSTPARTUM FILTRATION FRACTIONS

The slight diminution in filtration rate antepartum could result from afferent arteriolar constriction, which would cause a fall in filtration pressure, from edema of the kidney with resultant increase in interstitial pressure or from thickening of the basement membrane of the glomerulus with an increase in resistance to filtration (14). Any one of these mechanisms could conceivably be operative antepartum and disappear within a few days after delivery.

That the inulin clearance remains a true measure of glomerular filtration rate in toxemia is open to possible question on the ground that the permeability of the supposedly thickened glomerular filtering bed to the large inulin molecule might be reduced. In five subjects, however, on whom clearances of sorbitol or mannitol were done (15), the clearances of these hexitols were found to be identical with the simultaneous clearance of inulin.³ If inulin is not filtered at the same rate as

these smaller molecules, the hexitol/inulin clearance ratio can otherwise be maintained at the observed value of 1.0 only through the unlikely circumstance that a fraction of the filtered hexitol, exactly equal to that of the unfiltered inulin, is reabsorbed by the tubules. Yet tubular function in these five subjects is within normal limits as measured by diodrast T_m .

The observed alterations in effective renal blood flow are apparently not solely dependent upon the arterial blood pressure, for in some instances the diodrast clearance falls before the blood pressure falls. This observation indicates that the relatively high effective renal blood flow before delivery, and its decrease promptly thereafter, must be related to alterations in the renal vessels. The changes in effective renal blood flow may be explained by variations in efferent arteriolar tone (14), namely, dilatation of slight degree ante-

³ Hexitol and inulin clearances both antepartum and postpartum on patients F. P. (Table I), and H. F. and

M. S. (Table II), and postpartum on E. S. (Table I) and M. C. (Table II), have already been published by Smith, Finkelstein and Smith (15).

partum followed after delivery by a return to normal tone in the cured group and by spasm in the hypertensive group.

The dramatic improvement in the toxemic patient after delivery or fetal death is well known. The changes observed in diodrast clearances and filtration fraction—namely, a reduction from the level of normal renal blood flow or actual renal hyperemia to a level of renal ischemia accompanied by evidence of efferent arteriolar spasm—are seen soon after delivery. Both of these phenomena are evidently associated with the emptying of the uterus or the cessation of placental circulation. In those pregnant patients in whom hypertension is to persist and in whom the efferent hypertonus characteristic of essential hypertension is to appear after delivery, it is possible that constriction of the efferent arteriole is prevented by some unknown factor effective so long as pregnancy continues. Such an hypothesis is supported by the fact that in a small series of women with essential hypertension (unpublished data) renal blood flow is higher antepartum than postpartum and in two individuals is higher during pregnancy than before pregnancy. The filtration rate in these women with essential hypertension is unaffected by pregnancy.

In respect to the genesis of hypertension in toxemia of pregnancy, both in patients whose blood pressure is to return to normal and in those in whom hypertension is to persist, there is no evidence that renal ischemia plays any part. Indeed, when the blood pressure is highest, there is neither the renal ischemia nor the efferent arteriolar hypertonus characteristic of essential hypertension. Yet those patients in whom hypertension persists show in their postpartum clearances renal disturbances which cannot be distinguished from those of essential hypertension. The failure to find a reduction in renal blood flow in toxemia of pregnancy in which hypertension is such a striking feature is an argument against the theory that renal ischemia is a primary causal factor of the hypertensive process.

CONCLUSIONS

1. In toxemia of pregnancy the tubular excretory mass (diodrast T_m) is normal; the effective renal blood flow (diodrast clearance) is normal

or above normal; the glomerular filtration rate (inulin clearance) is somewhat reduced when referred to postpartum values; the filtration fraction is normal or low.

2. Following delivery the filtration fraction increases, in part because of a fall in diodrast clearance and in part because of an increase in inulin clearance. In the group with clinical cure these changes leave the figures within the normal range. In the group with persistent hypertension the results of these functional tests are identical with those found in essential hypertension.

3. The view that renal ischemia is an essential factor in the production of hypertension is opposed by the evidence that in the presence of the hypertension of toxemia there is a normal or even an increased renal blood flow.

We are indebted to Dr. Homer W. Smith for having many analyses of diodrast iodine carried out in the Department of Physiology before the method was set up in this Laboratory. We wish further particularly to express our thanks to him for his interest and advice.

BIBLIOGRAPHY

1. Goldring, W., *Lectures on Nephritis and Hypertension*. Edwards Brothers, Inc., Ann Arbor, Mich., 1937.
2. Smith, H. W., *The Physiology of the Kidney*. Oxford University Press, New York, 1937.
3. Smith, H. W., Goldring, W., and Chasis, H., The measurement of the tubular excretory mass, effective blood flow and filtration rate in the normal human kidney. *J. Clin. Invest.*, 1938, 17, 263.
4. Smith, H. W., Notes on the interpretation of clearance methods in the diseased kidney. *J. Clin. Invest.*, 1941, 20, 631.
5. Goldring, W., Chasis, H., Ranges, H. A., and Smith, H. W., Effective renal blood flow and functional excretory mass in essential hypertension. *J. Clin. Invest.*, 1938, 17, 505.
6. Goldring, W., Chasis, H., Ranges, H. A., and Smith, H. W., Effective renal blood flow in subjects with essential hypertension. *J. Clin. Invest.*, 1941, 20, 637.
7. Smith, H. W., *Physiology of the Kidney*. Porter Lectures. Series IX, Univ. Extension Div., Univ. of Kan., Lawrence, 1939.
8. Smith, H. W., Goldring, W., Chasis, H., and Ranges, H. A., Observations on the effective renal blood flow and functional excretory mass in man with special reference to essential hypertension. *Am. J. Physiol.*, 1938, 123, 189.
9. Chesley, L. C., Connell, E. J., Chesley, E. R., Katz, J. D., and Glisson, C. S., The diodrast clearance

- and renal blood flow in toxemias of pregnancy. *J. Clin. Invest.*, 1940, 19, 219.
10. Corcoran, A. C., and Page, I. H., Renal function in the late toxemias of pregnancy. *Am. J. M. Sc.*, 1941, 201, 385.
 11. Welsh, C. A., Wellen, I., and Taylor, H. C., Jr., The filtration rate, effective renal blood flow, tubular excretory mass and phenol red clearance in normal pregnancy. *J. Clin. Invest.*, 1942, 21, 57.
 12. Goldring, W., Chasis, H., Ranges, H. A., and Smith, H. W., Relations of effective renal blood flow and glomerular filtration to tubular excretory mass in normal men. *J. Clin. Invest.*, 1940, 19, 739.
 13. Chasis, H., Ranges, H. A., Goldring, W., and Smith, H. W., The control of renal blood flow and glomerular filtration in normal man. *J. Clin. Invest.*, 1938, 17, 683.
 14. Smith, H. W., Chasis, H., Goldring, W., and Ranges, H. A., Glomerular dynamics in the normal human kidney. *J. Clin. Invest.*, 1940, 19, 751.
 15. Smith, W. W., Finkelstein, N., and Smith, H. W., Renal excretion of hexitols (sorbitol, mannitol and dulcitol) and their derivatives (sorbiton, isomannide and sorbide) and of endogenous creatinine-like chromogen in dog and man. *J. Biol. Chem.*, 1940, 135, 231.

THE PREVENTION OF SENSORY NEURON DEGENERATION IN THE PIG, WITH SPECIAL REFERENCE TO THE RÔLE OF VARIOUS LIVER FRACTIONS¹

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In previous reports (1, 2) a description was given of abnormal gait and degeneration in the nervous system which developed in pigs under certain experimental conditions. It was suggested that these effects were the result of a dietary deficiency and evidence was presented which indicated that the changes took place in spite of adequate amounts of vitamin A, thiamin, riboflavin, nicotinic acid or vitamin E. Inanition or mineral deficiency did not seem to play any rôle. It was shown that a nerve-protecting factor was present in liver and, to a lesser extent, in yeast.

In an attempt to discover the nature of this protective factor or factors, further experiments have been carried out in which the basal deficient diet has been supplemented by various crude sources of accessory factors, chiefly various fractions of liver. More recently the newer vitamins, pyridoxine, pantothenic acid and choline have been used in conjunction with thiamin, riboflavin and nicotinic acid. It is the purpose of this report to give an account of the observations dealing with crude substances. Particular interest attaches to the nerve-protecting value of different fractions of liver, for degeneration similar to that observed in the pigs occurs in many cases of pernicious anemia in man. The fractions of liver used were those

produced during the manufacture of a liver extract which is effective in the treatment of pernicious anemia.

The literature dealing with "lameness," "stiffness" and "posterior paralysis" in swine and the available information regarding specific substances necessary for the preservation of the integrity of the nervous system, were reviewed completely in an earlier report (2). Full details were also given regarding the general plan of the experiment, the management of the animals, their housing and diet, and the methods of study. Only a brief account, therefore, need be given here.

GENERAL PROCEDURE

Pigs of approximately 3 weeks of age were shipped to Baltimore from the Beltsville Research Center of the United States Department of Agriculture. The animals were given a basic diet consisting of crude casein ("New Process," Sheffield By-Products Company) 9.5 grams, sucrose 21.0 grams, lard 4.0 grams and swine salt mixture Number 3 (2) 1.9 grams, per "unit." This amount ("one unit"), which furnishes 152 calories, or a fraction of a unit, was fed per kilogram body weight daily. The basic diet was supplemented with cod liver oil (Mead Johnson, 1800 units A and 175 units D per gram) 0.5 gram per kilogram body weight per day and powdered brewers' yeast (Mead Johnson) 3 grams per kilogram per day.

This diet appears to be adequate for the normal growth and development of young pigs. Weaning is usually accomplished satisfactorily although poor growth at first and even episodes of diarrhea may complicate the transition period. After the animals became accustomed to the diet, and once growth and general nutrition were satisfactory, they were divided into groups, usually consisting of 3 pigs each, composed of representatives of different litters which were as similar as possible as regards weight.

In all our experiments the neurological changes have been the same and have been confined to the sensory neuron, so that for the purpose of comparison they can be graded, as was done in our previous report (2). Briefly restated, the degrees of degeneration are classified as follows:

¹ Aided by grants from Parke, Davis and Company and the Rockefeller Foundation Fluid Research Fund of the Johns Hopkins Medical School, and carried out in co-operation with the Bureau of Animal Industry, United States Department of Agriculture.

Synthesized vitamins were furnished by Merck and Company, Incorporated, crude sources of vitamins by Mead Johnson and Company and Parke, Davis and Company. Liver fractions were prepared by Parke, Davis and Company.

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⁴ Fleischmann Fellow in Medicine.

0	No lesions.
1 or +	Definite changes in the peripheral nerves only: these consist in myelin degeneration with free fat granules and swollen neurilemmal cells. (When there is less extensive change in the peripheral nerves with only Marchi degeneration demonstrable, the lesion is sometimes graded as $\frac{1}{2}$ or \pm .)
2 or ++	The above changes are accompanied by chromatolytic changes in the cells of the posterior root ganglia.
3 or +++	Degeneration of the posterior roots and the root entry zone of the spinal cord, in addition to the ganglion cell and peripheral nerve changes already noted.

4 or ++++ Conspicuous degenerative changes in the nerves, ganglia, roots and posterior funiculi of the spinal cord.

PROTOCOLS: EXPERIMENT IV

Objects. (1) To gain further evidence regarding the protective value of whole desiccated liver and of brewers' yeast; (2) to determine whether the protective substance is present in wheat germ and alfalfa; (3) to obtain preliminary information regarding the protective value of the anti-pernicious anemia fraction of liver.

Conditions of experiment (Figure 1). Fifteen pigs derived from two litters were fed brewers' yeast (Mead

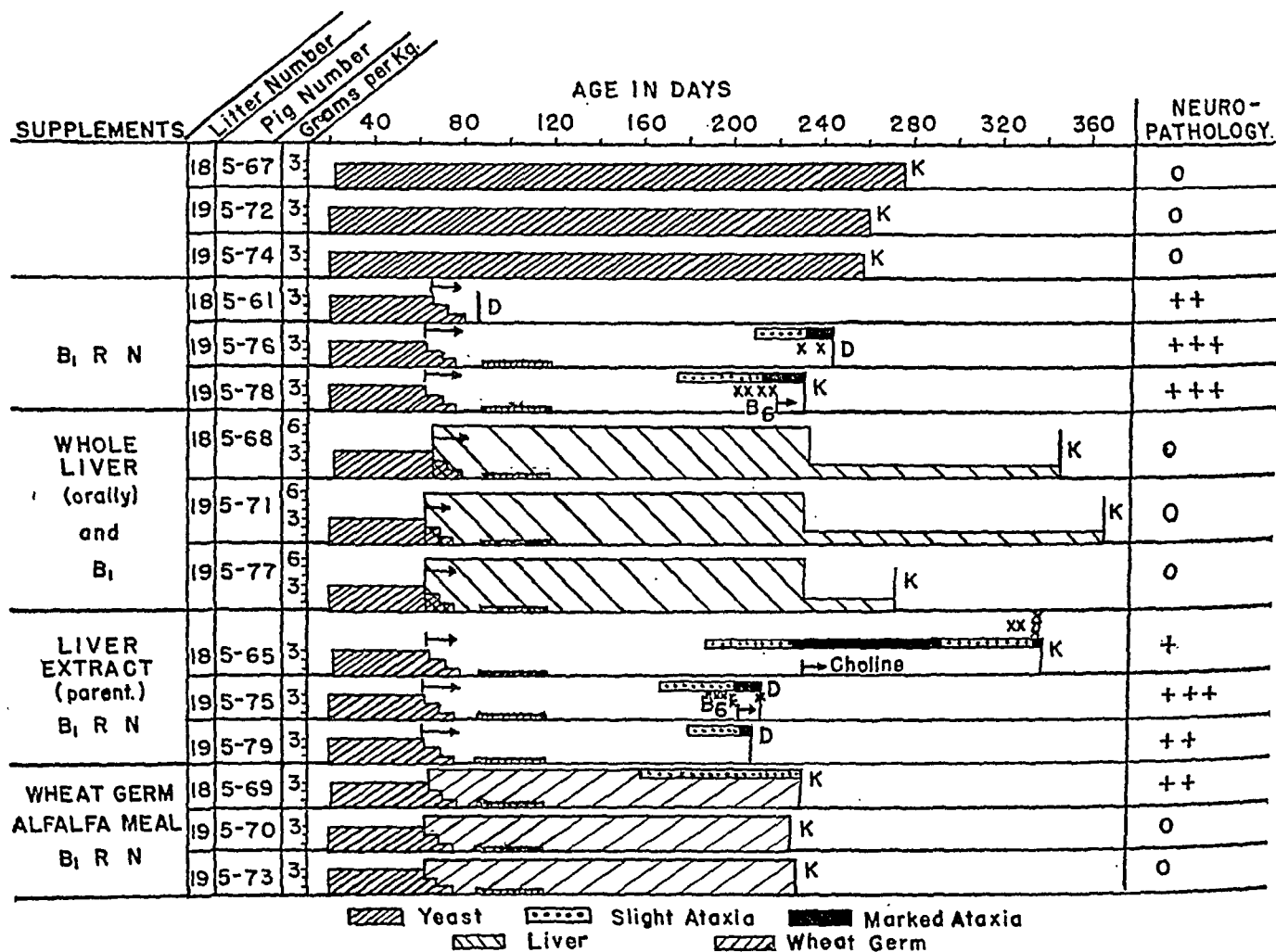


FIG. 1. EXPERIMENT IV. THE DEVELOPMENT OF "ATAXIA," CONVULSIONS AND NEUROPATHOLOGY IN PIGS GIVEN ONLY THIAMIN, RIBOFLAVIN AND NICOTINIC ACID IN THE PLACE OF SUBSTANCES CONTAINED IN YEAST AND IN WHOLE LIVER, COMPARED WITH THE COMPLETE PROTECTION AFFORDED BY YEAST OR LIVER

Liver extract, injected intramuscularly, gave no protection in the doses used and wheat germ plus alfalfa meal was less effective than whole liver or yeast.

All the pigs received the same basal diet. The amount of yeast and the length of time it was fed are shown. Supplements were started when yeast reduction commenced, as indicated by the arrows. For amounts see text. B₁ refers to thiamin; R to riboflavin; N to nicotinic acid.

The time of onset and severity of ataxia are indicated. The crosses mark the occurrence of convulsions. D indicates that the animal was found dead, K that it was sacrificed.

Pig 5-65 was given choline from the age of 233 days.

Johnson), 3 grams per kilogram body weight daily, until they were 63 to 67 days of age. In all but one group the amount of yeast fed was then gradually reduced so that after 13 days none was given. After a period of 15 days yeast was given again for 28 days in amounts of 0.5 gram per kilogram body weight daily. This was done in order to produce a less acute deficiency than would follow the sudden and complete withdrawal of yeast.

Supplements were added from the time the yeast was first reduced (63 to 67 days of age) (Table I and Figure 1). The quantities of thiamin, riboflavin and nicotinic acid given were the amounts estimated as present in the yeast which had been withdrawn; namely, thiamin 0.17 milligram, riboflavin 0.067 milligram, and nicotinic acid 0.4 milligram per gram of yeast.

Desiccated whole liver, which was fed as a supple-

TABLE I

Nature of supplement*	Pig number	Litter number	Sex	Age yeast reduction began	Time ensuing before "ataxia" began	Duration of experiment†	Age at death	Average daily gain in weight‡	Blood at time of death		Nature of death	Neuropathology¶
				days	days	days	days	grams	red blood cells, millions	MCV c.µ.		

EXPERIMENT IV												
B ₁ , R, N	5-61	18	M	65		22	87	40	6.10	55	Died. Rectal obstruction and prolapse of rectum.	++
B ₁ , R, N	5-76	19	F	64	111	180	244	156	4.98	48	Died (cause ?).	+++
B ₁ , R, N	5-78	19	F	64	112	169	232	178	4.65	56**	Killed.	+++
Des. liver, B ₁	5-68	18	F	66		281	347	346	9.35	55	Killed.	0
Des. liver, B ₁	5-71	19	M	64		304	368	433	7.40	61	Killed.	0
Des. liver, B ₁	5-77	19	F	64		209	273	567	7.10	64	Killed.	0
Parenteral liver ext., i.m., B ₁ , R, N†	5-65	18	M	66	117	275	341	173	8.45	45	Killed.	+
Parenteral liver ext., i.m., B ₁ , R, N	5-75	19	M	64	99	147	211	141	5.83	49**	Killed.	+++
Parenteral liver ext., i.m., B ₁ , R, N	5-79	19		64	120	147	211	143	5.35	43	Killed.	++
Wheat germ, alf., B ₁ , R, N	5-69	18	F	65	86	167	232	296	7.82	52	Killed. Cirrhosis.	++
Wheat germ, alf., B ₁ , R, N	5-70	19	M	64		166	229	482	8.95	54	Killed. Cirrhosis.	0
Wheat germ, alf., B ₁ , R, N	5-73	19	M	64		168	231	440	7.43	59	Killed. Cirrhosis.	0
Yeast continued	5-67	18	F	Not reduced			276	169†	7.40	56	Killed.	0
Yeast continued	5-72	19	M	Not reduced			259	297†	6.90	61	Killed.	0
Yeast continued	5-74	19	M	Not reduced			258	406†	6.90	63	Killed.	0

* B₁ refers to thiamin, R to riboflavin, N to nicotinic acid; "des. liver" is whole liver dried at a low temperature; parenteral liver extract is a crude anti-pernicious anemia liver extract (1 cc. = $\frac{1}{2}$ unit); alf. is alfalfa meal. Details concerning the various fractions of liver are given in the protocols of the experiments. Unless otherwise stated supplements were given orally; i.m. = intramuscularly.

† By "duration of experiment" is meant the period of time from the day on which yeast was reduced and supplements were started to the day of death. The average daily weight gain is calculated for this period. In the case of the 3 pigs in which the quantity of yeast was not reduced at any time, the average daily weight gain is calculated from the ages of 63 to 65 days to the day of death.

‡ Pig 5-65 received choline during the course of the experiment. See text.

§ Pig 5-96 received choline and pyridoxine (B₆) for a long period. See text.

|| Pig 6-00, at 246 days of age, received pyridoxine 50 mgm. intravenously, followed by the same dose the next day, and riboflavin 30 mgm. intravenously on the following day.

¶ For explanation of grading see "General Procedure."

** The blood values recorded are those found after convulsions had occurred and before pyridoxine was given. Following the administration of vitamin B₆ the red cell count of pig 5-78 was 4.93 million and the M.C.V. 61 c.µ; pig 5-75, R.B.C. 7.15, M.C.V. 51 c.µ; pig 6-00, R.B.C. 6.75, M.C.V. 57; pig 6-08, R.B.C. 6.68, M.C.V. 45.

M.C.V. is mean corpuscular volume.

TABLE I—Continued

Nature of supplement*	Pig number	Litter number	Sex	Age yeast reduction began	Time ensuing before "ataxia" began	Duration of experiment†	Age at death	Average daily gain in weight†	Blood at time of death		Nature of death	Neuropathology‡
				days	days	days	days	grams	red blood cells, millions	MCV c.μ.		
EXPERIMENT V												
Des. liver, B ₁ , R, N	5-88	20	M	94		264	358	241	6.90	59	Killed.	0
Des. liver, B ₁ , R, N	6-04	21	F	80		231	317	225	9.00	56	Killed.	0
Des. liver, B ₁ , R, N	6-09	22	M	76		272	348	155	8.60	55	Killed.	0
Press cake fraction, B ₁ , R, N	5-87	20	M	94	184	191	285	301	7.90	49	Died. Subdural hemorrhage and pulmonary edema.	+
Press cake fraction, B ₁ , R, N	6-03	21	F	80	193	196	276	186	5.48	45	Died (cause?).	+
Press cake fraction, B ₁ , R, N	6-08	22	M	80	177	216	292	207	4.31	48**	Killed. Lobar pneumonia.	++
"Whipple" fraction, B ₁ , R, N	5-89	20	M	94	72	93	187	151	4.55	55	Killed.	++
"Whipple" fraction, B ₁ , R, N§	5-96	20	F	94	98	213	307	145	5.40	59	Killed.	++
"Whipple" fraction, B ₁ , R, N	6-05	22	M	76	72	93	169	250	5.00	50	Killed.	++
"Cohn" fraction, B ₁ , R, N	6-00	21	M	80	198	267	347	232	5.33	47**	Died. Pulmonary congestion.	+
Parenteral liver ext., B ₁ , R, N	5-95	20	F	94		225	319	208	3.44	47	Died. Gastric hemorrhage.	0
Parenteral liver ext., B ₁ , R, N	5-99	21	M	80		181	261	243	6.40	47	Killed. Broncho-pneumonia.	+
Parenteral liver ext., B ₁ , R, N	6-14	22	F	76		272	348	240	7.13	59	Killed.	±
Permutit fraction, B ₁ , R, N	5-90	20	M	94	83	96	190	137	5.20	38	Killed. Chronic arthritis.	++
Permutit fraction, B ₁ , R, N	6-02	21	M	80	58	59	139	241	7.75	50	Died (cause?).	++
Permutit fraction, B ₁ , R, N	5-11	22	F	76	92	119	195	143	7.62	55	Killed.	+++
Mixture of fractions, B ₁ , R, N	5-92	20	M	94		138	222	258	7.18	50	Died. Rectal obstruction.	0
Mixture of fractions, B ₁ , R, N	5-98	21	M	80		257	337	391	7.25	66	Killed.	0
Mixture of fractions, B ₁ , R, N	6-12	22	M	76		260	336	246	7.40	59	Killed.	0
ADDITIONAL ANIMALS												
Parenteral liver ext., i.m., B ₁ , R, N	6-40	24	F	94	131	195	289	164	7.48	50	Killed.	++
Parenteral liver ext. (oral), B ₁ , R, N	6-50	24	M	94		191	285	108	4.77	44	Killed. Pleurisy. Arthritis.	+

ment to 3 pigs, was given in amounts of 6 grams per kilogram body weight per day for 169 days, after which time only 1.5 grams per kilogram were fed. The preparation used was hog liver, dried in vacuo at a low temperature (below 40° C.). One and one-half grams were

derived from 5 grams of fresh wet liver. Since the thiamin content of liver is lower than that of yeast, this vitamin was given, in the doses already mentioned, to those pigs receiving desiccated whole liver.

The anti-pernicious anemia liver extract used was a

crude extract (Parke, Davis) assayed at $\frac{1}{2}$ unit (U.S.P.) anti-anemic value per cc. It was given subcutaneously, 0.66 cc. per kilogram once a week for 15 weeks and then twice a week for the next 6 weeks. The one animal surviving beyond this time was given a total dose of 20 cc. twice weekly until it was killed. This represented an average amount of 1.0 cc. per kilogram per week.

Wheat germ (Scaltest Laboratories) was fed in amounts of 6 grams per kilogram per day and alfalfa meal 5 grams per kilogram per day.

Ascorbic acid, 16 milligrams per kilogram, was given orally three times a week throughout their lifetime to all the pigs in this experiment except pigs 5-67, 5-72 and 5-74.

Results (Figure 1, Table I). Confirming our previous observations, abnormal gait and extensive degeneration in the nervous system developed in the pigs which were given only thiamin, riboflavin and nicotinic acid in addition to the basal diet, cod liver oil and ascorbic acid. On the other hand, desiccated whole liver plus thiamin afforded protection even in animals observed for a very long time. The period of observation in the animals which were given yeast as the only supplement was not as long as in the case of those given liver but it was equal to that of the animals given only thiamin, riboflavin and nicotinic acid. The gait of the animals fed only yeast supplement was normal in all instances in Experiment IV and no histologic changes could be found at autopsy.

It will be noted that the record of wheat germ plus alfalfa meal, given in addition to thiamin, riboflavin and nicotinic acid, is not as good as that of desiccated whole liver or yeast, for one animal developed incoordination in gait and degenerative changes were found in the nervous system. It is of interest to note that cirrhosis of the liver was found in all 3 of these animals but was absent in the rest of our pigs. Cirrhosis of the liver has been described as a manifestation of nutritional deficiency in rabbits (3).

The anti-pernicious anemia fraction of liver, given parenterally in addition to thiamin, riboflavin and nicotinic acid, which were administered orally, failed, in the doses used, to produce a result significantly better than that in animals given these vitamins alone.

Choline chloride, 10 mgm. per kgm. per day, was given orally to pig 5-65 167 days after yeast had been replaced by thiamin, riboflavin and nicotinic acid, given orally, and parenteral liver ex-

tract, given intramuscularly. A well pronounced slapping gait had been present for two months and this animal's experimental mates had already died following the development of ataxia and other signs of deficiency, including convulsions in one of them (Figure 1). The ataxia did not progress once the administration of choline was started. Weight gain occurred and, after 2 months, definite improvement in gait was noted. Some improvement in the general appearance of the animal also took place and after almost 3 months of choline administration no abnormality in gait could be noted. This improvement did not persist, however. For a period of 3 weeks a slight abnormality in gait was perceptible, then definite ataxia appeared and in another week an epileptiform convulsion occurred. Following this the gait deteriorated considerably and repeated convulsions took place. The animal was finally sacrificed.

The growth and general development of the animals fed liver, wheat germ and alfalfa meal, or yeast, were good, while in the remainder they were less satisfactory (Table I). In the pigs given only thiamin, riboflavin and nicotinic acid, and in those given the anti-pernicious anemia fraction of liver in addition to these vitamins, a generally untidy appearance, diarrhea and loss of appetite occurred; in several even vomiting accompanied the onset of abnormal gait. The vomiting and diarrhea ceased after several days, possibly as the result of the administration of 11 grams of yeast daily for several days, but the untidy appearance persisted. Later diarrhea recurred in several animals in episodes of 3 or 4 days' duration and disappeared spontaneously.

Epileptiform convulsions were observed in 2 of the 3 pigs receiving thiamin, riboflavin and nicotinic acid as the only supplements and in 2 of the 3 animals receiving injections of liver extract in addition to thiamin, riboflavin and nicotinic acid by mouth. The single member of the first group in which convulsions were not observed (5-61) died at a very early age. The convulsions first appeared when the pigs were 197 to 329 days of age, and after the experiment had been in progress 137 to 263 days.

Preceding the convulsion it was usually noted that the pig was excited and "nervous." Further excitement such as the rattle of the food pans or that produced by allowing the pig into a runway

in order to observe its gait would sometimes precipitate an attack. Frequently the pig would run at great speed up and down the runway and then would collapse. The convulsions varied in intensity but in general the pattern was as follows: the pig lay on its side, all four limbs and the muscles of the body jerked rapidly, the head was held in extension, the eyes were shut or turned upwards and saliva drooled from the mouth. After several minutes, the spasmodic muscular contractions ceased and a stage of stupor followed which also lasted several minutes. Occasionally a gurgling sound could be heard. When the stupor was over the pig would try to get up and, when it finally succeeded, it would proceed in a staggering, dazed fashion. An animal previously able to run at a furious pace would stumble and fall repeatedly. In several animals ataxia appeared for the first time following a convulsion and progressed rapidly from then on.

Once convulsions set in they were often repeated at intervals of several days, sometimes more often (Figure 1). Pig 5-78, following the fifth convulsion, was given pyridoxine hydrochloride, 100 mgm. intravenously, followed the next day by the same dose together with 30 mgm. thiamin chloride. During the following 11 days the same dose of thiamin, together with 10 mgm. pyridoxine, was given daily intravenously. No further convulsions were observed but the animal remained very clumsy and ataxic. Pig 5-75, following six convulsions in the same number of days, was helpless and unable to stand. Pyridoxine, 100 mgm., was given daily intravenously for the remaining 8 days before the pig died. The state of helplessness was unchanged, but no further convulsions were observed until the day of death when one more was noted.

In pig 5-65 convulsions appeared at a much later date than in the other pigs. This animal differed from the others in that it was given choline for a long time, as already described. Five convulsions were observed occurring in rapid succession in the 13 days preceding death.

Severe anemia (Figure 2) was observed in 2 of the pigs given only thiamin, riboflavin and nicotinic acid (5-76, 5-78) and in 2 given parenteral liver

extract in addition (5-75, 5-79). The first 2 animals developed anemia at the same time that other signs of deficiency appeared (untidy appearance, loss of appetite, diarrhea, vomiting). Following the administration of a very small amount of yeast (0.5 gram per kgm. daily for 11 days) the anemia was promptly relieved (Figure 2). After 3 weeks anemia recurred and gradually became more severe than it was originally. Following the administration of pyridoxine and thiamin to pig 5-78, a slight decrease of anemia of questionable significance occurred.

The anemia in the 2 pigs given parenteral liver extract was steadily progressive and terminally it developed very rapidly. One of these animals, 5-75, was given pyridoxine. The doses were larger than those given 5-78, and a prompt and substantial rise in the blood count followed (Figure 2).

As the anemia developed, increasing anisocytosis but practically no poikilocytosis or hypochromia appeared. A few polychromatophilic macrocytes were noted, but the most striking change was the appearance of microcytes, some extremely small. When the anemia became quite severe, a well marked decrease in the mean corpuscular volume was found.

EXPERIMENT V

Object. To obtain further information regarding the nature of the factor or factors in whole liver which protect the nervous system of the pig.

Conditions of experiment. In the process of manufacture of liver extract for the treatment of pernicious anemia, hog liver is digested at pH 5, then heated at 85° C. and filtered. The residue is known as the filter press cake and ordinarily is discarded. The filtrate, after reduction to small volume at a low temperature in vacuo, is treated with alcohol added in amounts to make a 70 per cent concentration. A precipitate forms which is sometimes known as the Whipple "secondary anemia" fraction. The clear fluid obtained after removal of the 70 per cent alcohol precipitate by filtration is known as the Cohn anti-pernicious anemia fraction. This can be desiccated as is done to obtain a dry preparation for oral administration in the treatment of pernicious anemia or it may be passed through permutit in order to prepare a fraction which can be given parenterally. The process can be outlined schematically as follows:

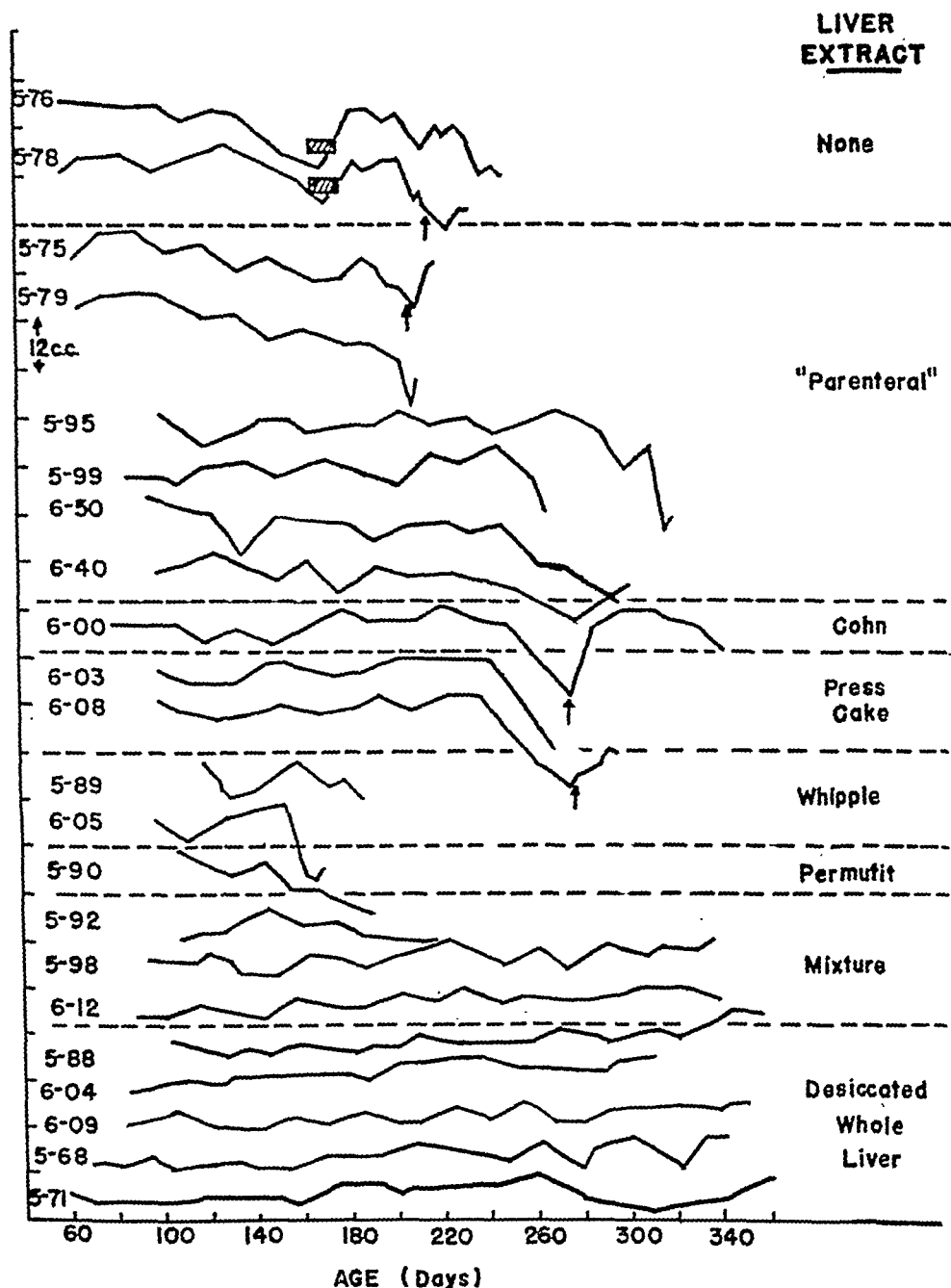


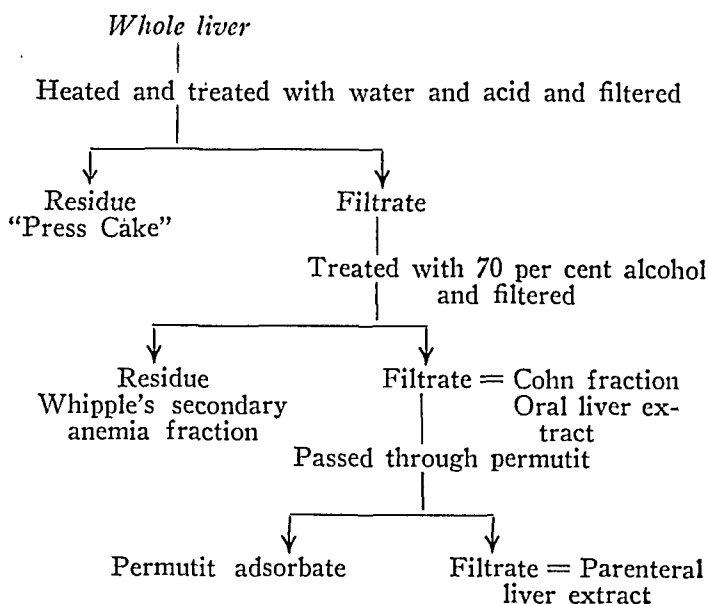
FIG. 2. THE DEVELOPMENT OF ANEMIA IN PIGS FED AN ARTIFICIAL DIET SUPPLEMENTED WITH THIAMIN, RIBOFLAVIN AND NICOTINIC ACID AND VARIOUS LIVER EXTRACTS

The continuous lines represent volume of packed red cells in cubic centimeters per 100 cubic centimeters of blood. The interrupted lines divide the various groups. The arrows represent the time of administration of pyridoxine in pigs 5-75 and 6-08 and of pyridoxine together with thiamin in 5-78 and with riboflavin in 6-00. The hatched areas represent the temporary administration of yeast to pigs 5-76 and 5-78.

Note the absence of anemia, as indicated by the essentially horizontal lines, in pigs fed the mixture of liver extracts or desiccated whole liver.

(For brevity, curves for the following pigs, none of which showed a significant degree of anemia, have been omitted: 5-77, desiccated whole liver; 5-65 and 6-14, parenteral liver extract; 5-87, press cake; 5-96, Whipple fraction; 6-02 and 6-11, permutit fraction; 5-67, 5-72 and 5-74, brewers' yeast; and 5-69, 5-70 and 5-73, wheat germ and alfalfa meal.)

Each division on the ordinate represents 12 cc. of packed red cells per 100 cc. blood.



In order to assay these fractions, 21 pigs, weaned as already described, were divided at ages of 69 to 87 days into 7 comparable groups of 3 each. Yeast was withdrawn completely over a period of 2 weeks. From the time of the first reduction of yeast, the administration of thiamin, riboflavin and nicotinic acid, in the doses indicated in Experiment IV, and of whole liver or liver fractions, was commenced. The latter were given in the form of a dry powder mixed with the basal diet each day. All supplements were given orally.

For the purpose of this experiment a large amount of liver was processed in the plant of Parke, Davis and Company and all fractions were saved. The following are the amounts of the various fractions obtained *per 5 grams fresh whole liver*:

Desiccated whole liver	1.560 grams
"Press cake"	0.915 gram
"Whipple" fraction	0.215 gram
"Cohn" fraction	0.200 gram
Parenteral liver extract	0.180 gram
Permutit adsorbate	0.095 gram

The amount of any fraction derived from 5 grams of fresh whole liver will be referred to here as one "equivalent." This amount of whole liver, per kilogram body weight, corresponds to the quantities of whole liver used at one time in the treatment of pernicious anemia (300 to 350 grams per patient per day).

One of the above six products was fed to each of a group of 3 pigs. At the start of the experiment the daily dose per kilogram was one equivalent in the case of the desiccated whole liver and two equivalents in the case of the various fractions. The double dose of the fractions was used because of the possibility that protective factors might have been lost in the process of manufacture. A seventh group of pigs was given the press cake, Whipple, parenteral liver extract and permutit fractions mixed in the proportions derived from 5 grams of fresh liver. These animals received 1.41 grams of the mixture per kilogram, or one equivalent.

As shown in Figure 3, the quantities of the liver fractions used were altered in the course of the experiment. Modifications were made in terms of "equivalents" of fresh liver and parallel changes were made in all groups.

Two of the 3 pigs fed the Cohn fraction died suddenly following a bout of diarrhea shortly after the experiment was commenced and have therefore been excluded from this report.

Results (Figure 3 and Table I). It was evident very early that the permutit fraction was lacking in essential growth factors and in factors needed for the protection of the nervous system. The same was true of the Whipple fraction. In the other groups of animals, on the other hand, no evidence of deficiency appeared at this period. The amounts of the press cake, Cohn, and parenteral liver extract fractions were therefore reduced from two to one "equivalent." This change was maintained for 56 days when a further reduction to one-half equivalent was made. Shortly after this reduction, evidence of abnormal gait appeared in the animals receiving press cake and in the pig fed the Cohn fraction. Since it was feared that the amounts of the extracts had been reduced to so low a level that abnormal gait might develop in all groups and that differences between them might thereby be obscured, 4 weeks after this last change the amount of supplement was increased to one equivalent. No additional animals developed abnormalities in gait, but 2 of those receiving the parenteral liver extract were found on histologic examination to have demonstrable changes of mild degree in the nervous system. None of the pigs receiving the mixture of fractions or desiccated whole liver showed signs of incoordination or evidence of nerve lesions histologically.

The pigs fed desiccated whole liver and the mixture of liver extracts, respectively, grew rapidly (Figure 4). Those in the latter group looked particularly well developed and well nourished. The Cohn, parenteral liver extract and press cake fractions in the order named, were less satisfactory in promoting growth. A pronounced deficiency of growth factors was observed in the pigs fed the Whipple and the permutit fractions.

One of the pigs (5-96) fed the Whipple fraction was given choline chloride in the same doses as those used for pig 5-65 (Experiment IV). After 3 weeks pyridoxine, 0.3 mgm. per kgm. daily, was also given. This animal lived consider-

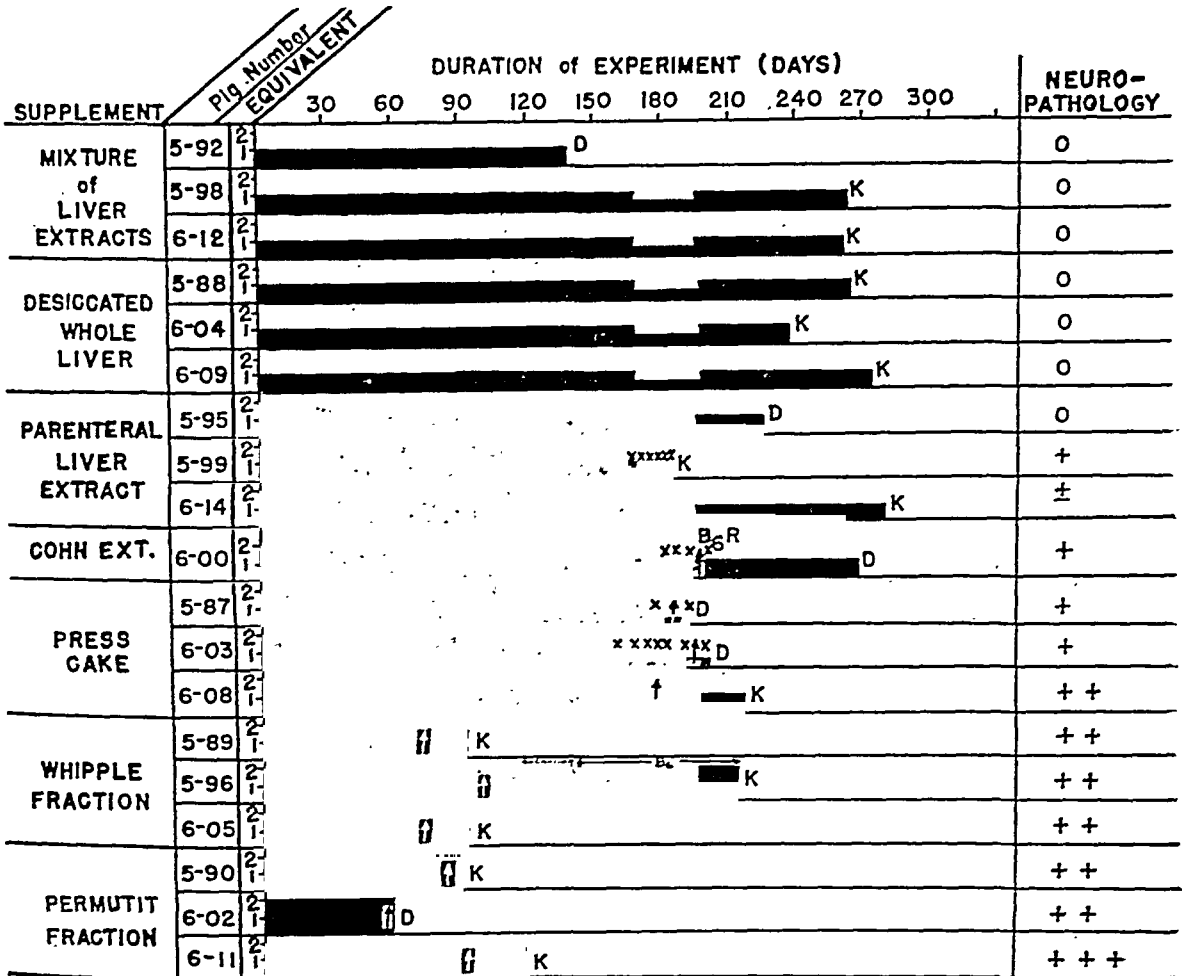


FIG. 3. EXPERIMENT V. THE RELATION OF "ATAXIA," CONVULSIONS AND NEUROPATHOLOGY TO THE AMOUNT AND TYPE OF LIVER EXTRACT FED

Each "equivalent" of a liver extract is the amount derived from 5 grams of fresh, whole liver. For further details see Table I and protocol. All pigs received thiamin, riboflavin and nicotinic acid.

The vertical arrows indicate the approximate date of onset of ataxia. The crosses represent convulsions. D indicates that the animal was found dead; K that it was sacrificed.

Pig 5-96 was given choline and pyridoxine during the period indicated.

ably longer than its mates, but no consistent improvement in gait occurred. There was no further weight gain and finally the animal deteriorated and became helpless. It was sacrificed 94 days after choline was first given.

Epileptiform convulsions, like those already described, were observed in 2 of the 3 pigs fed press cake, in one of those fed the parenteral liver extract and in the animal receiving the Cohn fraction (Figure 3). The last was the only pig treated with vitamins. This animal had had four observed convulsions and had reached a state of

complete helplessness. Pyridoxine was given, 50 mgm. intravenously on 2 successive days. No additional convulsions were seen. However, there was only slight improvement in the degree of prostration and therefore on the third day 30 mgm. riboflavin were given intravenously. Three days later the animal was able to move about actively, the only abnormality present being the stamping gait characteristic of our "ataxic" animals.

Severe anemia was observed in 2 out of 3 pigs receiving the press cake fraction (Figure 2 and Table I). The third (5-87) developed microcy-

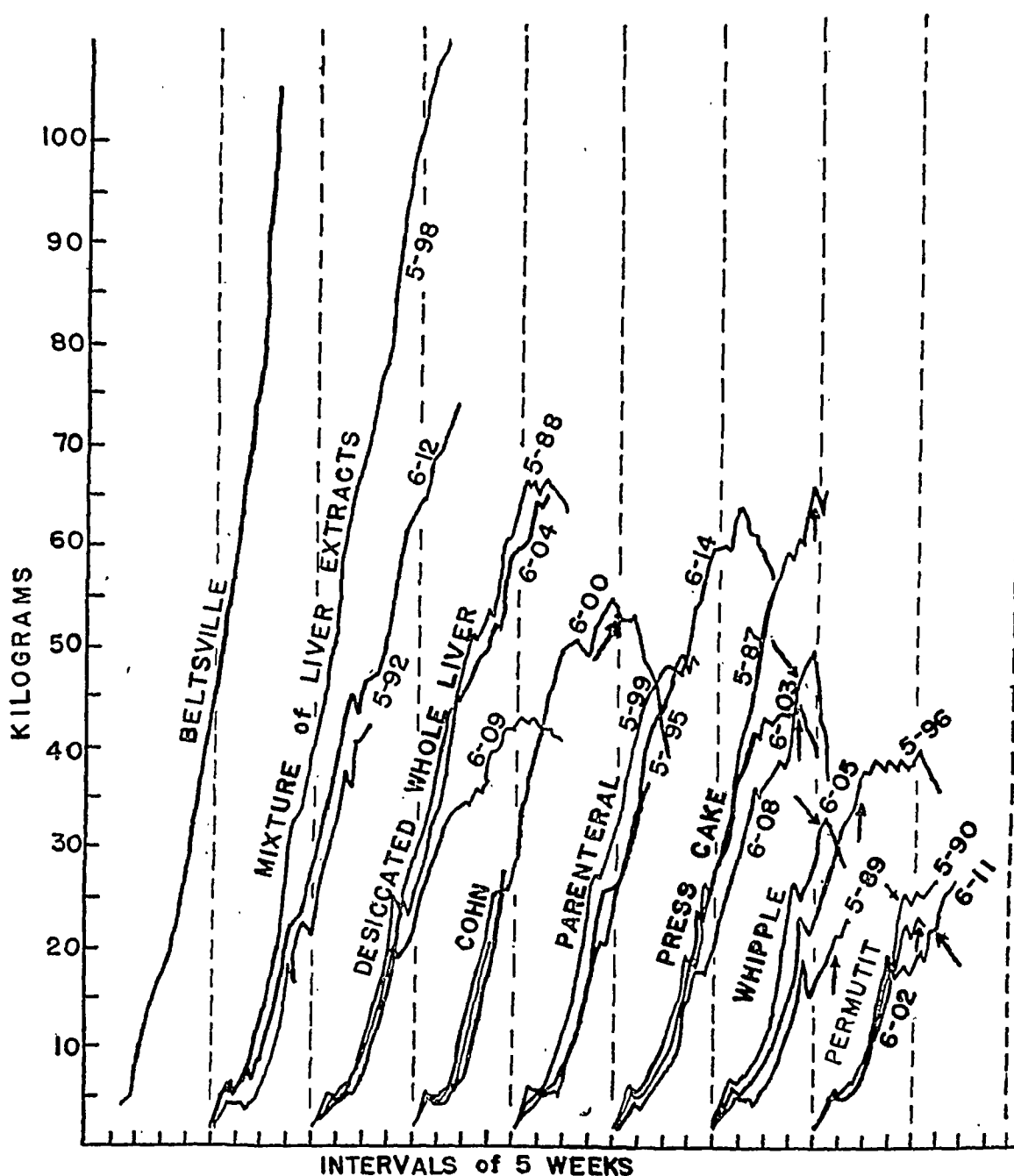


FIG. 4. GROWTH OF PIGS RECEIVING DESICCATED WHOLE LIVER, VARIOUS LIVER EXTRACTS AND A MIXTURE OF THESE EXTRACTS, COMPARED WITH THAT OF PIGS RAISED AT THE EXPERIMENTAL FARM OF THE U. S. DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND.

Arrows indicate the onset of ataxia.

All pigs received one unit of diet (152 calories) per kgm. until they were 4½ to 5 months of age, half a unit for the next 6 to 8 weeks, a third unit for the following 6 to 8 weeks, a half unit again for 4 weeks and a third of a unit thereafter.

tosis without severe anemia. One of these pigs (6-08) was treated with pyridoxine (50 mgm. daily for 16 days) and a rise in red cell count without increase in the size of the cells followed. This pig's feet were ulcerated and bleeding, making interpretation of the results difficult. Anemia was present also in 2 pigs receiving the Whipple

fraction. The third member of this group (5-96) developed no anemia. This pig differed from its mates in that it was given choline and pyridoxine in addition to the liver fraction. In the single pig fed the Cohn fraction (6-00) severe anemia and microcytosis developed. Following two injections of pyridoxine (50 mgm. each), together with one



FIGS. 5-13. ABNORMAL GAIT IN VARIOUS PIGS AND COMA FOLLOWING CONVULSION IN FIG 5-65

Figures 5, 6 and 7. Pig 5-96 ("Whipple" fraction of liver). In Figure 6, note the "goose-step," in Figure 7 the attempt to balance on two legs. Also note the malnutrition and the untidy appearance of the hair (Figure 5) so characteristic of pigs suffering from nutritional deficiency. These are enlargements from a motion picture film.

Figure 8. Pig 5-65 (parenteral liver extract) in a comatose state following a convulsion.

Figures 9 and 10. Pig 5-65, 2 weeks previously, showing the difficulty in controlling the fore and hind legs.

Figures 11, 12 and 13. Pig 6-05 ("Whipple" fraction of liver), showing the attempt to gain support by leaning against a pen (Figure 11), the swaying of the body on turning into the pen (Figure 12) and the groping motion of the left hind leg before it was set down on the ground (Figure 13).

injection of riboflavin (30 mgm.), the reticulocytes increased from 4.2 per cent to 11.4 per cent on the fourth day following the first injection and the anemia was relieved in 6 days. Of the group receiving "parenteral" liver extract, anemia was observed in 2 out of 3 pigs. In one of these gastric hemorrhage occurred and accounted at least in part for the anemia. Severe anemia was found in only one of the pigs fed the permutit fraction of liver.

DISCUSSION

Protection of the nervous system. For the period of the experiments, the development of degenerative changes in the nervous system was prevented by feeding desiccated whole liver or brewers' yeast. Wheat germ together with alfalfa meal was more variable in effect. Desiccated whole liver gave protection in amounts derived from 5 grams fresh liver per kilogram body weight daily. The combination of fractions obtained from this quantity of liver was equally effective but none of the individual fractions, fed singly, gave complete protection. It should be pointed out that all the animals described here except those given yeast throughout the experiment received supplements of thiamin alone or thiamin, riboflavin and nicotinic acid in amounts adequate to prevent deficiencies of these substances.

The extract of liver which is used in the treatment of pernicious anemia was clearly the best of the extracts assayed. None of the 3 pigs receiving the parenteral fraction in Experiment V developed an abnormal gait. One was found free of histologic nerve changes and one showed peripheral nerve lesions detectable only by Marchi staining. Only one animal was found to have changes demonstrable by the Pal-Weigert or the Mahon stains for myelin.

Thus the pigs developed relatively mild neuropathological changes and no observed ataxia when fed one-half to one equivalent of the anti-pernicious anemia fraction per kilogram daily. This is in contrast to the more extensive neurological lesions, as well as definite ataxia, which occurred in animals given less (approximately one-tenth equivalent) of a similar fraction parenterally (Experiment IV). Even the latter dose, however, is large when compared with the amounts effective on parenteral administration in relieving anemia

in man. On the basis of this evidence, it appears that large amounts of the anti-pernicious anemia fraction are required by the pig.

The antianemic effect of liver extract in pernicious anemia is greatly enhanced by parenteral administration of the extract. In order to compare the value of "parenteral" liver extract when given orally and parenterally, respectively, 2 additional pigs (6-40 and 6-50, Table I) were given per kilogram body weight per day that amount of extract derived from 2 grams fresh liver. The experimental conditions were the same as those already described and thiamin, riboflavin and nicotinic acid were fed as in the animals already discussed. The liver extract was injected intramuscularly twice a week in one pig while the other pig received the same amount orally with its food twice a week. After 3 weeks the dose of extract was increased 50 per cent in both animals. Pig 6-40 finally received as much as 70 and even 80 cc. liver extract intramuscularly twice a week.

These quantities of liver extract, which were less than those fed the pigs in Experiment V, failed to protect the nervous system of pigs 6-40 and 6-50. The animal receiving injections grew more rapidly than its mate. The latter was thin and untidy in appearance, while the former looked well-nourished. However, an abnormal gait developed in pig 6-40 in spite of the appearance of good health. Ataxia was not observed in the pig fed liver extract. Histologically the latter animal showed a less severe degree of degeneration in the nervous system than pig 6-40.

It thus appears that there is no increase in the efficacy of liver extract in protecting the nervous system of young pigs when it is given parenterally.

Convulsions. Convulsions were observed in our animals after 5 or 6 months on a deficient diet. They occurred when only thiamin, riboflavin and nicotinic acid represented the "B" vitamins or when "parenteral" liver extract, the Cohn fraction or the press cake fraction was furnished in addition to these vitamins. The cause of the convulsions is not altogether clear. One pig which died after a convulsion (5-87) was found to have a subdural hemorrhage, but in the other animals no anatomical lesions in the brain have been discovered in the sections studied so far.

That the convulsions were a manifestation of

nutritional deficiency seems apparent for they have never been observed in animals receiving whole desiccated liver, a mixture of liver extracts which probably is equivalent to whole liver, brewers' yeast or wheat germ and alfalfa meal in addition to thiamin, riboflavin and nicotinic acid. Epileptiform convulsions have been observed in pigs (4), dogs (5, 6) and rats (7) and have been attributed to pyridoxine deficiency.

Anemia. The anemia observed in our animals was no doubt due to nutritional deficiency for in those fed whole liver, yeast, wheat germ and alfalfa or the mixture of liver fractions, the blood was maintained at an essentially constant level. Why anemia was more pronounced in one pig fed a deficient diet than in another, or why a few on deficient diets failed to develop any anemia, is not evident. The anemia observed was similar to that described by others in dogs (8) and pigs (4) and has been attributed to pyridoxine deficiency.

Gastric analyses were performed in most of our pigs from time to time. No significant and consistent abnormalities were found.

Relation to pernicious anemia. Little can be added to what was stated in a previous publication (1) regarding the possible relationship of the degenerative changes in the nervous system of these pigs and those observed in some cases of pernicious anemia. The fact that the fraction of liver which is effective in relieving the anemia in the latter disease was more valuable in protecting the nervous system of pigs than the other liver fractions, is of interest. That large amounts of liver extract were necessary for this purpose is another point of similarity with pernicious anemia, for it is a generally held clinical impression that large quantities of liver or of crude liver extracts are required to effect any improvement or to prevent the advance of lesions in the nervous system of patients. Unlike the blood picture in pernicious anemia, macrocytic anemia was not observed in our animals.

It remains to be proved that the changes in the nervous system observed in the pigs and those seen in pernicious anemia are due to the same cause. If they are, then perhaps our work provides evidence that the factors protecting the nervous system and the antianemic substance are distinct from each other because we failed to produce macro-

cytic anemia on a diet deficient in the nerve-protecting factor. Assays are being made of the antianemic potency of the livers of our animals by which it is hoped to obtain information regarding the relation between the antianemic and the nerve-protecting factors.

Perhaps the strongest evidence to support the view that there is little relationship between the factors concerned with blood formation and with maintaining the integrity of the nervous system is the fact that in a number of human disorders characterized by macrocytic anemia, and which respond to liver therapy, such as sprue, degenerative changes in the nervous system are scarcely ever found. It may or may not be significant that pernicious anemia differs from such conditions in which neurologic symptoms are rare in that a profound disturbance in gastric secretion is characteristic of pernicious anemia. This would suggest that the gastric juice may play a rôle in preparing for the body a substance necessary for maintaining the integrity of the nervous system. The observation that liver extract given parenterally seemed somewhat less effective in protecting the nervous system of the pigs than was liver extract given by mouth may, if it is confirmed, suggest a need for its passage via the gastro-intestinal tract and may point again to the difference between the nerve-protecting and the antianemic factors in liver extract. In man, the antianemic factor is 30 to 60 times as potent when given intramuscularly as compared with its effect when given by mouth.

SUMMARY

1. Abnormal gait and degenerative changes in peripheral nerves, spinal ganglia and the posterior funiculi of the spinal cord developed in pigs deficient in factors other than thiamin, riboflavin and nicotinic acid but did not occur when desiccated whole liver or brewers' yeast was fed.⁵

⁵ In an article which has just appeared (Mitchell, D., Spinal cord degeneration produced by dietary means. Brain, 1941, 64, 165) failure to prevent the development of demyelination of the spinal cord in pigs by the feeding of yeast is reported. The suggestion is made that the pathological changes in the nervous system observed by Mitchell and by ourselves are due to a lack of some essential inorganic micro-constituent of the diet, probably copper. No experimental evidence to support the latter

Wheat germ together with alfalfa meal was not as effective in preventing sensory neuron degeneration as liver or yeast.

2. The various fractions obtained during the manufacture of anti-pernicious anemia liver extract were assayed for their value in protecting the nervous system. The fraction used in the treatment of pernicious anemia was the most effective of all those tested but relatively large amounts were required. The value of the extract was not increased by parenteral administration.

3. Growth was better when the anti-pernicious anemia extract or the "press cake" fraction was given than when other fractions were used, but was not as satisfactory as when the whole dried liver or a mixture of all the fractions was fed. Furthermore, convulsions and anemia developed in pigs which were fed these fractions.

4. It should be pointed out that all the animals described here except those given yeast throughout the experiment received supplements of thiamin alone or thiamin, riboflavin and nicotinic acid in

view is given. Mitchell fed the salt mixture described in our preliminary experiments (1), not that used in later studies (2) or in those reported here, which contains significant and probably adequate amounts of copper as well as other metals (2). This, as well as consideration of the experiments reported by Mitchell, makes it appear more probable that his failure to prevent the development of degenerative changes in the nervous system is attributable to a lack of necessary protective factors in the yeast he used. In our earliest experiments, Experiments I and II (2), the feeding of yeast prevented the development of pathological changes only in 5 out of 12 animals. This led us to change the source of the yeast. Since type "M" (Mead, Johnson and Company) has been fed from the time pigs are received, all animals have been protected successfully (Experiment III (2) and IV (in the present report)). Experimental evidence to be reported shortly indicates that the protective substance in yeast and in liver is not copper.

amounts adequate to prevent deficiencies of these substances.

The cooperation of Dr. E. A. Sharp of Parke, Davis and Company, Dr. D. F. Robertson of Merck and Company, Incorporated, and Dr. Warren M. Cox, Jr., of Mead Johnson and Company, and their associates is gratefully acknowledged. Dr. Richard H. Follis, Jr., kindly examined some of the pathological sections. Messrs. Stewart Humphreys, William Thaler, Adolph Suksta, and Edward Sullivan and Mrs. Lottie Lowenstein, Miss Marjorie Lauritsen and Miss Ruth Herringman rendered valuable technical assistance.

BIBLIOGRAPHY

1. Wintrobe, M. M., Mitchell, D. M., and Kolb, L. C., Sensory neuron degeneration in vitamin deficiency. *J. Exper. Med.*, 1938, 68, 207.
2. Wintrobe, M. M., Miller, J. L., Jr., and Lisco, H., The relation of diet to the occurrence of ataxia and degeneration in the nervous system of pigs. *Bull. Johns Hopkins Hosp.*, 1940, 67, 377.
3. Rich, A. R., and Hamilton, J. D., Experimental production of cirrhosis of liver by means of deficient diet. *Bull. Johns Hopkins Hosp.*, 1940, 66, 185.
4. Chick, H., Macrae, T. F., Martin, A. J. P., and Martin, C. J., The water-soluble B-vitamins other than aneurin (vitamin B₁), riboflavin and nicotinic acid required by the pig. *Biochem. J.*, 1938, 32, 2207.
5. Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H., Production of microcytic hypochromic anemia in puppies on synthetic diet deficient in rat antidermatitis factor (vitamin B₆). *J. Nutrition*, 1938, 16, 197.
6. Street, H. R., Cowgill, G. R., and Zimmerman, H. M., Some observations of vitamin B₆ deficiency in the dog. *J. Nutrition*, 1941, 21, 275.
7. Chick, H., El Sadr, M. M., and Worden, A. N., Occurrence of fits of an epileptiform nature in rats maintained for long periods on a diet deprived of vitamin B₆. *Biochem. J.*, 1940, 34, 595.
8. Fouts, P. J., Helmer, O. M., and Lepkovsky, S., Nutritional microcytic hypochromic anemia in dogs cured with crystalline factor I. *Am. J. M. Sc.*, 1940, 199, 163.

PATHOLOGICAL VARIATIONS IN BLOOD AND SPINAL FLUID PYRUVIC ACID¹

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Pyruvic acid is a normal intermediary of carbohydrate metabolism (1, 2). It has been previously demonstrated that thiamin, or more particularly thiamin pyrophosphate (cocarboxylase), is concerned in the normal catabolism of pyruvic acid (1). In Oriental beri-beri (3) and peripheral neuropathy in the alcohol addict (4)—both diseases associated with a deficiency of thiamin—hyperpyruvemia does occur.

Pyruvic acid is a keto acid and therefore a bisulfite binding substance. Previous investigators (5, 6, 7) have frequently used the measurement of the total bisulfite binding substances (B.B.S.) as an indication of the pyruvic acid level of the blood. More recent work (8, 9, 10, 11) indicates that this is not justified and that a more specific method for pyruvic acid must be used.

Utilizing such a method, we have determined the concentration of pyruvic acid in 60 normal subjects (12). The figures varied from 0.77 to 1.16 mgm. per cent, the average being 0.98 mgm. per cent. We have considered as abnormally high cases with blood pyruvic acid levels above 1.30 mgm. per cent. The spinal fluid pyruvate is 70 to 120 per cent of a corresponding blood sample (average 82 per cent) (13).

The present study was undertaken in order to determine the clinical significance of the pyruvic acid levels in blood and cerebrospinal fluid.

MATERIALS AND METHODS

Pyruvic acid was determined in the blood by a method previously described (12, 14). Spinal fluid pyruvate was determined similarly, except that it was found that the stabilizing medium (monoiodoacetate) was unnecessary. All samples

¹ Aided by grants from the John and Mary R. Markle Foundation, the Williams-Waterman Fund of the Research Corporation, and an Anonymous Donor for Research in Psychosomatic Medicine.

were obtained in fasting subjects at complete bed rest.

RESULTS

(A) Normal individuals

Blood pyruvic acid was determined in 41 normal individuals (internes, laboratory workers, attending physicians). The figures varied from 0.77 to 1.23 mgm. per cent (average 1.02).

(B) "Acute" peripheral neuropathy

Blood determinations were done on 48 individuals. The figures ranged from 1.00 to 3.63 mgm. per cent (average 1.88). Three had figures below 1.30 mgm. per cent, the accepted normal. In 23 of these individuals, spinal fluid pyruvic acid determinations were done. The figures ranged from 1.00 to 2.37 mgm. per cent (average 1.64). We (15) have previously indicated that all these cases of "acute" peripheral neuropathy were associated with some type of cortical dysfunction. This group included 11 cases of Wernicke's syndrome, a disease known to be associated with a deficiency of thiamin (16, 17), and the others showed various types of encephalopathy, including Korsakoff syndrome, delirium tremens, nicotinic acid deficiency and other less serious types of cortical dysfunction. In 3 individuals, the "acute" peripheral neuropathy was associated with beri-beri heart disease.

(C) Chronic peripheral neuropathy

These cases include (a) those who had received adequate vitamin therapy without a complete return to normal functioning of the peripheral nerves, or (b) individuals who had been on hospital diets for some time.

Blood determinations were done on 25 such cases with figures ranging from 0.85 to 1.31 mgm.

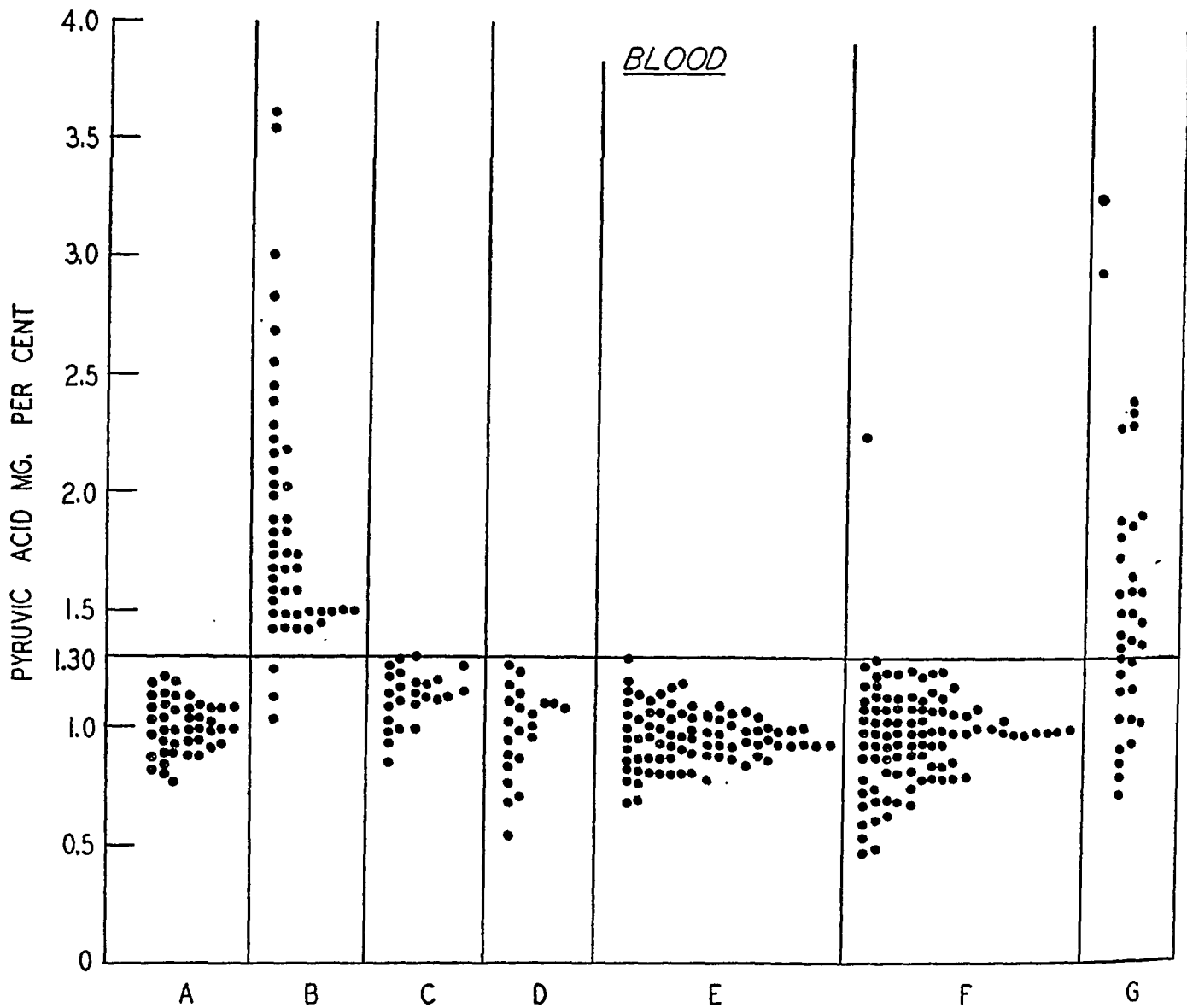


FIG. 1. BLOOD PYRUVIC ACID

- A. Normal individuals
- B. "Acute" peripheral neuropathy
- C. Chronic peripheral neuropathy
- D. Chronic alcoholics with no objective evidence of nutritional deficiency
- E. Psychiatric disorders
- F. Medical disorders (no febrile reaction)
- G. Fever

per cent (average 1.12). In 10 of these cases, pyruvic acid was determined in the spinal fluid. The figures ranged from 0.83 to 1.31 mgm. per cent (average 1.07).

(D) Chronic alcoholics with no objective evidence of nutritional deficiency

Blood pyruvic acid was determined on 22 such individuals. The figures ranged from 0.54 to 1.27 mgm. per cent (average 0.99). In 13 of these individuals, the spinal fluid pyruvate ranged from 0.60 to 1.26 mgm. per cent (average 0.96).

(E) Psychiatric disorders with no objective evidence of nutritional deficiency

Determinations were done on the following:

Diagnostic grouping	Blood Number of cases	Spinal fluid Number of cases
Schizophrenia	22	24
Manic-depressive (depressed)	2	4
Involuntal melancholia	6	1
Reactive depression	7	1
Behavior disorders in children	27	13
Behavior disorders associated with mental deficiency	5	8
Psychopathic personality	8	2
Psychoneurosis	5	0

All figures in these groups fell within the normal range. The blood figures varied from 0.70 to 1.29 mgm. per cent; the spinal fluid from 0.42 to 1.22 mgm. per cent.

(F) *Medical disorders (no febrile reaction)*

Determinations were done on the following:

Diagnosis	Blood Number of cases	Spinal fluid Number of cases
Generalized arteriosclerosis	16	4
Hypertensive vascular disease	14	0
Diabetes mellitus	11	0
Central nervous system syphilis	10	5
Mild cardiac failure (not agitated or dyspneic)	10	0
Chronic diffuse glomerulonephritis	4	0
Paroxysmal convulsive disorder	3	3
Pellagrous stomatitis (non-alcoholic)	2	0
Cirrhosis of liver	2	0
Pernicious anemia	2	0
Bronchial asthma	2	0
Submersion (aspiration pneumonia)	2	0
Primary brain tumor	0	2

In addition, we studied the blood pyruvic acid in one case each of congenital cerebral aneurysm, generalized syphilis, hyperthyroidism, birth injury, arthritis, cor pulmonale, drug addiction, insulin coma, lysol poisoning, arsenical poisoning, bromide poisoning, barbiturate poisoning, carcinoma of the prostate, bronchopneumonia, lead neuropathy, Little's disease, congenital aphasia, multiple sclerosis, spinal cord tumor, cortical atrophy, meningo-encephalitis of undetermined etiology. Spinal fluid pyruvate was determined in one case each of hyperthyroidism, Little's disease, and arsenical poisoning. The figures in the blood and cerebrospinal fluid were normal in every instance but one. This was a case of lymphoepithelioma of the pharynx with associated cachexia. Blood pyruvic acid in this case was 2.32 mgm. per cent. Determination of vitamin B₁ in the blood yielded an abnormally low figure (1.82 gamma).

Mention must also be made of a paper by Yanof (18) who reports blood pyruvate levels of 3 mgm.

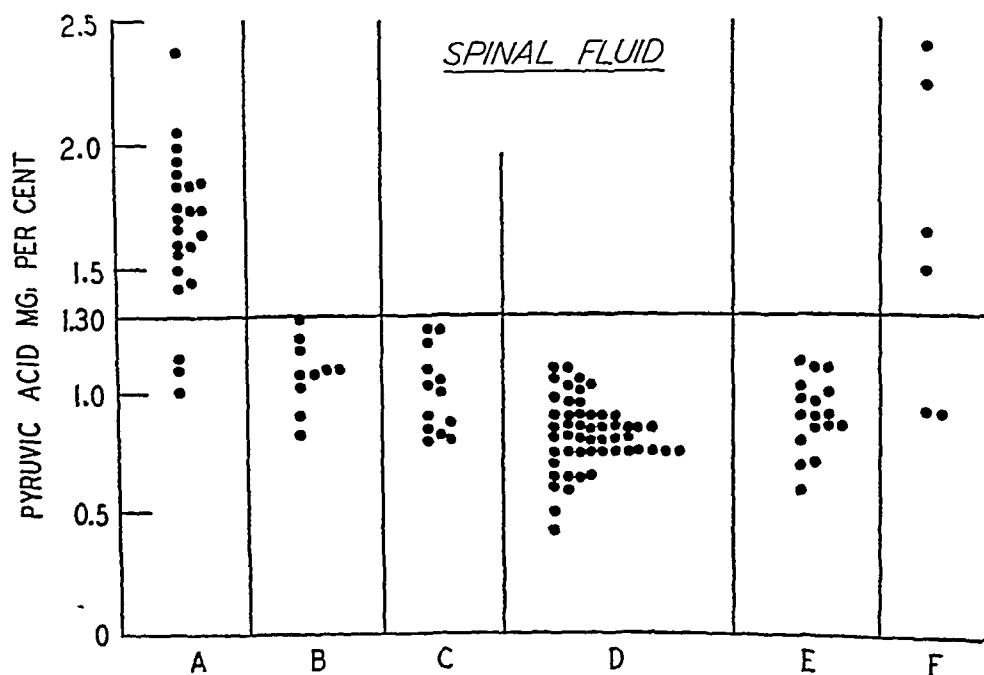


FIG. 2. SPINAL FLUID PYRUVIC ACID

- A. "Acute" peripheral neuropathy
- B. Chronic peripheral neuropathy
- C. Chronic alcoholics with no objective evidence of nutritional deficiency
- D. Psychiatric disorders
- E. Medical disorders (no febrile reaction)
- F. Fever

per cent in cases of decompensated heart disease. These figures may be the result of (1) agitation and increased muscular activity and (2) glycolysis following hypoxemia. That the elevated figures obtained by the author are not invariably associated with cardiac decompensation as such is evidenced by our findings in 10 non-agitated cases of mild cardiac failure.

(G) *Fever*

These cases represent a very heterogeneous grouping, the common factor being an elevation of temperature of varying degree and duration. They include cases of meningitis, pneumonia, carbon-monoxide poisoning, cerebral accidents, therapeutic malaria, erysipelas, etc. Thirty-five blood determinations were done, of which 22 were above the accepted normal (1.30 mgm. per cent). In 6 instances, spinal fluid pyruvate determinations were done, and in 4 of these the figures were definitely elevated.

DISCUSSION

It will be noted that two groups of cases showed an abnormal elevation of blood and spinal fluid pyruvates: (a) those associated with nutritional deficiency (45 cases of "acute" peripheral neuropathy and 1 case of lymphoepithelioma with associated cachexia), and (b) 22 cases associated with an elevation in temperature. The first group is readily explained by the fact that thiamin, or more particularly thiamin pyrophosphate, is necessary for the normal catabolism of pyruvic acid. Since it is now generally accepted that the "acute" peripheral neuropathy of alcohol addicts is associated with a deficiency of thiamin, this defect in pyruvic acid metabolism becomes readily understandable (4). In addition, we have presented evidence suggesting that pyruvic acid is a normal intermediary of carbohydrate catabolism in man (2, 14). In normal individuals, there is a significant rise of pyruvic acid after the ingestion of glucose, reaching a maximum at the end of the first hour and returning to normal within three hours (19). In 14 cases of "acute" peripheral neuropathy, the pyruvic acid curve following glucose ingestion was abnormally elevated and prolonged (14). The maximum rise in blood pyruvate was not only greater than that seen in normal indi-

viduals, but the elevation above the fasting level was maintained for a period of at least four hours. Furthermore, the maximum rise did not occur at the end of the first hour, as seen in normals, but the pyruvate continued to rise in every instance, and maximal figures were obtained at the end of the second, third or fourth hours. In addition, we have observed that thiamin therapy, alone or in conjunction with other members of the vitamin B complex, corrects this defect in pyruvic acid metabolism (20).

On the other hand, we have noted hyperpyruvemia in 22 cases of hyperpyrexia unassociated with any clinical evidence of vitamin deficiency. In these cases it may be that the elevation in total metabolism so increases the thiamin requirements that a deficiency occurs with resultant hyperpyruvemia. On the other hand, it should be noted that none of these cases had peripheral neuropathy. This suggests that either the metabolic disturbance must exist for some time before peripheral neuropathy occurs or that hyperpyruvemia may be related to metabolic disturbances other than thiamin deficiency (21). This subject is now under investigation.

We also wish to emphasize that a normal fasting pyruvic acid may not be an invariable evidence of thiamin adequacy. In 1 case of "acute" peripheral neuropathy and 2 cases of "chronic" peripheral neuropathy with normal fasting blood pyruvate, we have observed an abnormal pyruvate curve following glucose ingestion. This suggests that the pyruvic acid curve following glucose ingestion may prove to be a more sensitive index of thiamin adequacy than the fasting blood pyruvic acid level.

SUMMARY AND CONCLUSIONS

1. Normal fasting pyruvic acid levels are reported in the blood and spinal fluid in cases of "chronic" peripheral neuropathy, chronic alcoholism without objective evidence of nutritional deficiency, various psychiatric and medical disorders and certain cases of hyperpyrexia.

2. Hyperpyruvemia has been noted in cases of "acute" peripheral neuropathy, and in about two-thirds of the cases associated with hyperpyrexia.

3. The possible clinical significance of pyruvic acid determinations in the blood and spinal fluid is discussed.

BIBLIOGRAPHY

1. Banga, I., Ochoa, S., and Peters, R. A., Pyruvate oxidation in brain; some dialyzable components of the pyruvate oxidation system. *Biochem. J.*, 1939, 33, 1980.
2. Bueding, E., Stein, M. H., and Wortis, H., The formation of pyruvic acid following glucose ingestion in man. *J. Biol. Chem.*, 1941, 139, 793.
3. Lu, G. D., Studies on metabolism of pyruvic acid in normal and vitamin B₁ deficient states. *Biochem. J.*, 1939, 33, 249.
4. Wortis, H., Bueding, E., and Jolliffe, N., Pyruvic acid studies in the peripheral neuropathy of the alcohol addict. *New England J. Med.* (In press.)
5. Banerji, G. G., and Harris, L. J., Methods for assessing the level of nutrition; a carbohydrate tolerance test for vitamin B₁. Part I, Experiments with rats. *Biochem. J.*, 1939, 33, 1346.
6. Platt, B. S., and Lu, G. D., Chemical and clinical findings in beriberi with specific reference to vitamin B₁ deficiency. *Quart. J. Med.*, 1936, 5, 355.
7. Thompson, R. H. S., and Johnson, R. E., Blood pyruvate in vitamin B₁ deficiency. *Biochem. J.*, 1935, 29, 694.
8. Robinson, W. D., Melnick, D., and Field, H., Jr., Correlation between the concentration of bisulphite binding substances in the blood and urinary thiamin excretion. *J. Clin. Invest.*, 1940, 19, 483.
9. Wilkins, R. W., Weiss, S., and Taylor, F. H. L., Effect and rate of removal of pyruvic acid administered to normal persons and to patients with and without "vitamin B deficiency." *Ann. Int. Med.*, 1939, 12, 938.
10. Wortis, H., Bueding, E., and Wilson, W. E., Bisulfite binding substances (B.B.S.) in the blood and cerebrospinal fluid. *Proc. Soc. Exper. Biol. and Med.*, 1940, 43, 279.
11. Wortis, H., Bueding, E., and Wilson, W. E., The clinical significance of bisulfite binding substances (B.B.S.) in the blood and cerebrospinal fluid. *Am. J. Psychiat.*, 1940, 97, 573.
12. Bueding, E., and Wortis, H., The stabilization and determination of pyruvic acid in the blood. *J. Biol. Chem.*, 1940, 133, 585.
13. Bueding, E., and Wortis, H., Pyruvic acid in the blood and cerebrospinal fluid. *Proc. Soc. Exper. Biol. and Med.*, 1940, 44, 245.
14. Bueding, E., Stein, M. H., and Wortis, H., Pyruvic acid curves following glucose ingestion in normal and thiamin deficient subjects. *J. Biol. Chem.*, 1941, 140, 697.
15. Wortis, H., and Jolliffe, N., The present status of the vitamins in nervous health and disease. *New York State J. Med.*, 1941, 41, 1461.
16. Alexander, L., Wernicke's disease; identity of lesions produced experimentally by B₁ avitaminosis in pigeons with hemorrhagic polioencephalitis occurring in chronic alcoholism in man. *Am. J. Path.*, 1940, 16, 61.
17. Jolliffe, N., Wortis, H., and Fein, H. D., The Wernicke syndrome. *J. Nerv. and Ment. Dis.*, 1941, 93, 214.
18. Yanof, Z. A., Blood pyruvic acid in heart disease. *Proc. Soc. Exper. Biol. and Med.*, 1941, 47, 516.
19. Bueding, E., Wortis, H., Stein, M. H., and Jolliffe, N., Pathological variations in blood pyruvic acid. *J. Clin. Invest.*, 1941, 20, 441.
20. Wortis, H., Bueding, E., and Stern, M., Unpublished observations.
21. Wortis, H., and Bueding, E., The clinical significance of pyruvic acid content of blood and cerebrospinal fluid. *Tr. Am. Neurol. A.*, 1940, 66, 90.

THE SIGNIFICANCE OF PORPHYRINURIA IN LEAD POISONING¹

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A pathologic increase in the amount of porphyrin excreted in the urine by patients with lead poisoning was first reported by Garrod (1) in 1892. Since then it has been generally assumed that this porphyrinuria is related in some way to the destruction of hemoglobin. Until recently there has been no means of testing the validity of this belief. Lately, however, the advance in knowledge concerning the chemistry of the porphyrins, and the development of methods for distinguishing one type from another, have provided a new approach towards an understanding of the underlying metabolic disorder responsible for the porphyrinuria of plumbism.

The isomeric identity of the Type III coproporphyrin found in the urine in lead poisoning with the Type III protoporphyrin of hemoglobin has been demonstrated by Grotepass (2) and by Watson (3). The porphyrin in the urine in lead poisoning thus fundamentally differs from the Type I isomer which is the predominant type excreted in several other conditions associated with porphyrinuria. These facts justify the assumption that the porphyrinuria in lead poisoning is a consequence of some alteration in the metabolism of hemoglobin itself. In theory, it might originate from an altered synthesis of hemoglobin just as probably as from hemoglobin destruction; but owing to the traditional belief that the anemia in lead poisoning is hemolytic in nature, only the latter possibility has received any general attention until recently. The hypothesis that the coproporphyrin excreted in lead poisoning is a product of hemoglobin destruction requires, however, an additional corollary. In pathologic conditions as-

sociated with excessive hemoglobin destruction, the breakdown products are eliminated in the bile in the normal manner without any large increase in the amount of Type III porphyrin excreted in the urine. Therefore, if the increased output of coproporphyrin in lead poisoning is conceived to be on the basis of increased destruction of hemoglobin, it is necessary to postulate that one effect of lead is to cause a partial interference in the pathway by which hemoglobin is normally broken down to bilirubin. Thus, it must be assumed that part of the destroyed blood pigment is diverted from the bile and appears in the urine with its original ring structure still intact.

In view of these considerations, it seemed of interest to study the mechanism of hemoglobin destruction in patients with lead poisoning. Evidence of some defect in this process would lend support to the hypothesis that the porphyrin appearing in the urine in this condition is derived from the breakdown of hemoglobin. On the other hand, failure to obtain such evidence would furnish additional support for the more recent theory that this porphyrinuria is a consequence of defective hemoglobin synthesis.

The experiments described in this paper were carried out on two patients suffering from lead poisoning with anemia and porphyrinuria. An attempt was made to trace the path of hemoglobin destruction in these patients by observing the effect of injected hemoglobin on the production of bilirubin and on the excretion of coproporphyrin and urobilinogen in the urine and feces.

METHODS AND MATERIAL

During the period of investigation the patients were confined to bed and, in order to reduce the possibility of dietary porphyrins contributing to the estimated porphyrin excretion, partook of a meat, fish, and egg-free diet. All urine and feces were collected in dark glass containers and kept in an icebox with toluol as a preservative. In successive 24-hour specimens of urine the coproporphyrin was measured by a modification of the method of Brugsch and Keys (4) previously described by us (5). The fecal

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coproporphyrin excretion was measured on aliquots from successive 3-day accumulations of the stools by a modification of the same method which is described in detail below. The urobilinogen excreted in the urine and feces was estimated by Watson's (6) method. Plasma and urinary hemoglobin were estimated by Bing and Baker's (7) modification of Wu's method; and plasma bilirubin by Barron's (9) method. Hemoglobin solutions were prepared for intravenous injection by the method of Ottenberg and Fox (8).

The estimation of ether-soluble porphyrin in the feces

The 3-day collection of feces is weighed as collected and transferred to the beaker of an electric mixer. The large lumps are broken down with a glass stirring rod, and water is gradually stirred in to bring the volume to a given amount (usually 1000 ml.). The feces and water are thoroughly mixed into a paste by means of an electric stirrer and a measured volume, equivalent to 10 grams of the original weight of the stool, is taken out with a measuring cylinder and transferred to a mortar. The paste is then repeatedly ground up with 1 to 2 ml. glacial acetic acid and 20 to 40 ml. of ether. The acetic acid-ether extracts are decanted into a brown half-liter bottle and, finally, when no more color can be extracted from the stool by the acetic acid-ether mixture, the stool is also added to the contents of the bottle. The bottle is then shaken on a machine for 2 hours. The acetic acid-ether and feces mixture is filtered. The residue on the filter paper is mixed with more acetic acid, further ether is added and the filtration repeated. The two filtrates are then combined.

The total acetic acid-ether extract is washed six times with water in a separatory funnel, drained and extracted three times on the rotary machine with 10 ml. lots of 5 per cent HCl solution. A final extraction is made with 10 ml. of 10 per cent HCl. The HCl extracts are combined and neutralized to Congo red by the addition of sodium acetate crystals. The neutral solution is repeatedly extracted with 10 ml. amounts of acetic acid and 100 ml. amounts of ether until there is no red fluorescence visible in the acetic acid-ether layer when examined by ultraviolet light.

The acetic acid-ether extracts are combined, washed six times with water, drained and reextracted with 5 per cent and 10 per cent HCl as previously. This whole procedure is repeated three times but during the final HCl extraction only 5 per cent HCl is used.

The combined 5 per cent HCl extracts are now shaken repeatedly with 5 ml. chloroform until the chloroform layer shows no red fluorescence when held in front of the ultra-violet lamp. The acid fraction is now diluted to 0.05 per cent HCl by the addition of distilled water and again repeatedly extracted with 5 ml. amounts of chloroform. These steps are introduced to remove any protoporphyrin which may be present. The 0.05 per cent HCl extract is then made neutral to Congo red by the addition of sodium acetate crystals and the neutral solution repeatedly extracted with acetic acid and ether as

previously. The combined acetic acid-ether extracts are washed, drained and reextracted with 5 ml. amounts of 5 per cent HCl. The combined HCl extracts are made up to a suitable volume and read in the same manner as the final HCl extracts obtained from the urine. The per diem excretion of porphyrin in the feces can be calculated by using the following equation:

$$\frac{\text{Total weight of 3-day collection of feces}}{10} \times \frac{\text{Final volume of HCl extract}}{\text{Volume of HCl extract used for reading against standard}} \times \frac{\text{The reading in micrograms}}{3} = \text{daily excretion of porphyrin in micrograms.}$$

By this method the excretion of porphyrin in normal individuals varied from 80 to 280 micrograms per diem.

The two patients selected for study each possessed the characteristic features of lead poisoning.

Case 1. Case 1 was a 24-year-old white male who had been employed in polishing soldered metal surfaces. He was admitted to the hospital following an attack of colic and complained of weakness, nervousness and constipation. His gums showed a well-marked lead line, but there was no evidence of neuritis. His hemoglobin was 54 per cent (Sahli), red blood cells 2.73 million per cubic millimeter, hematocrit 25 per cent, reticulocytes 9.6 per cent with 1 or 2 stippled cells visible in each high powered field. The blood lead was 0.007 mgm. per 10 grams of blood (normal 0.002 mgm.).⁴

During his stay in the hospital, the urine was consistently normal in color, yet contained about 1000 micrograms of coproporphyrin in each 24-hour collection (Figure 1). The normal urinary excretion of coproporphyrin, as measured by the method employed, usually ranges between 20 and 40 micrograms, and rarely exceeds 96 micrograms in 24 hours. The daily fecal coproporphyrin excretion of Case 1 averaged about 200 micrograms. The urine never contained more than a trace of urobilinogen, and the fecal excretion of urobilinogen was always within normal limits.

It is interesting to note that the urinary excretion of coproporphyrin was unaffected by the administration of 500 mgm. of nicotinic acid or of 5 U.S.P. units of liver extract daily for 12 days (Figure 1). This therapy was administered in view of a published report (10) which claims that nicotinic acid reduces the porphyrinuria in lead poisoning.

⁴ The blood lead determination was very kindly performed by Dr. A. J. Plummer of the Evans Memorial Hospital.

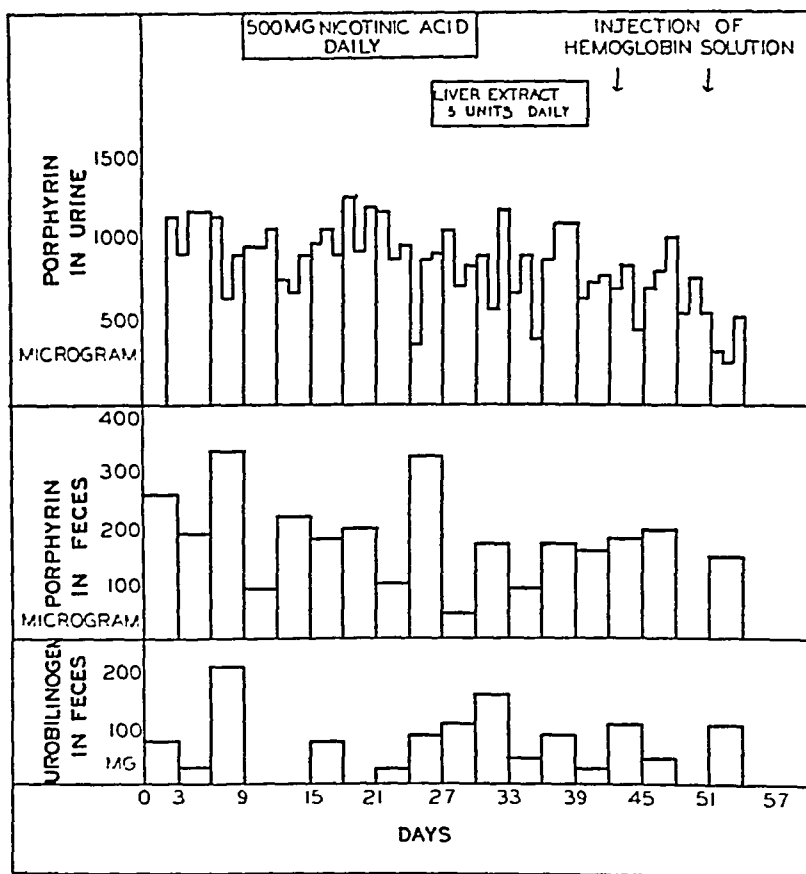


FIG. 1. CASE 1. EXCRETION OF COPROPORPHYRIN IN URINE AND FECES, AND FECAL EXCRETION OF UROBILINOGEN BEFORE AND AFTER TWO INJECTIONS OF ABOUT 7.5 AND 19 GRAMS OF HEMOGLOBIN, RESPECTIVELY

Case 2. Case 2 was a 40-year-old colored male lead smelter. He had been suffering from colic prior to admission and was constipated, nervous and "shaky." A definite lead line was present in the gums. No evidence of neuritis was noted. The hemoglobin was 60 per cent (Sahli), red blood cells 3.9 million per cubic millimeter, hematocrit 38 per cent, reticulocytes 6.7 per cent, stippled cells 1.8 per cent.

The urine was normal in color⁵ throughout his stay in the hospital but contained an average of 1200 micrograms of coproporphyrin in each 24-hour collection (Figure 2). The urinary excretion of urobilinogen varied from a trace to 4 mgm. a day. The amount of copro-

porphyrin in the feces was at first somewhat increased, averaging 450 micrograms a day for the first 9 days, but later fell to within normal limits. The excretion of urobilinogen in the feces was always within the limits of normal.

RESULTS

After a suitable control period in each case, solutions of hemoglobin were injected intravenously during a period of 30 minutes.

Case 1. In the first test, about 7.5 grams of hemoglobin derived from 50 ml. of normal blood were injected. This amount of hemoglobin, if totally destroyed, would yield theoretically about 250 mgm. of protoporphyrin. Following the injection, hemoglobin was detectable in the plasma and reached a peak of 55 mgm. per cent after one hour. The plasma bilirubin rose from a control level of 0.38 to a peak of 0.70 per cent in 4 hours. There was, therefore, a maximum increase of 0.32

⁵ We wish to emphasize that in both the cases reported here the urine was entirely normal in appearance despite its abnormal content of porphyrin. It is evident that the port wine coloration, traditionally associated with porphyrinuria, is not found in lead poisoning owing to the absence of other associated pigments which are usually present where the urine contains excessive amounts of porphyrin. This question will be discussed more fully in a later communication.

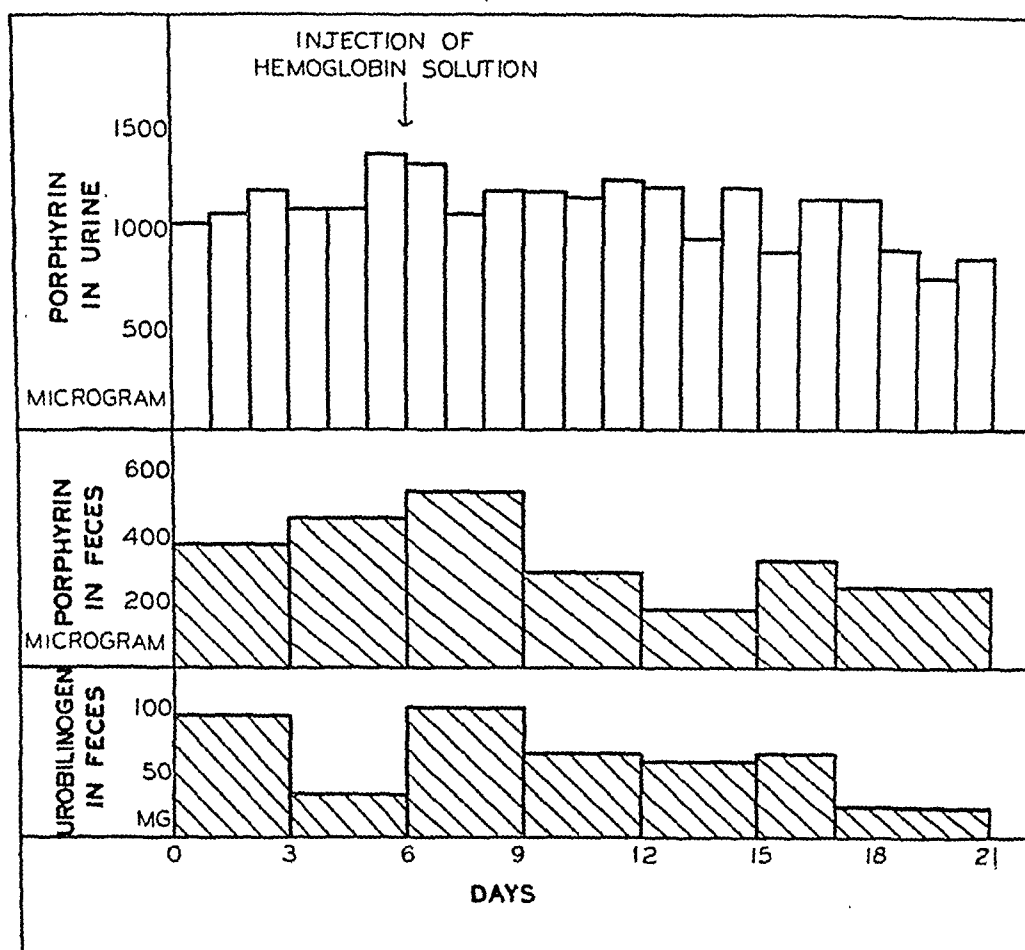


FIG. 2. CASE 2. EXCRETION OF COPROPORPHYRIN IN URINE AND FECES, AND FECAL EXCRETION OF UROBILINOGEN BEFORE AND AFTER AN INJECTION OF ABOUT 7.9 GRAMS OF HEMOGLOBIN

mgm. of bilirubin per 100 ml. of plasma. Assuming the blood volume to have been approximately 5 liters, with the observed cell volume 25 per cent, about 12 mgm. of bilirubin derived from the injected hemoglobin must have been present in the circulating blood stream during the height of its production. It is fair to assume, therefore, that *at least* this amount of the 250 mgm. of protoporphyrin present in the injected hemoglobin was converted to bilirubin; yet none appeared as additional coproporphyrin in the urine. The urinary excretion of coproporphyrin was not increased even by 100 micrograms, an amount that would certainly have been detected by the method of estimation employed.

The fecal excretion of urobilinogen and coproporphyrin remained unchanged during the test (Figure 1) as did the urinary excretion of urobilinogen.

The second test on Case 1 was even more definitive. About 19 grams of hemoglobin derived

from 125 ml. of normal blood and equivalent to 630 mgm. of protoporphyrin were injected intravenously. The maximum increase in plasma bilirubin following the injection was 2.8 mgm. per 100 ml. of plasma (Figure 3), from which the calculated maximum quantity of circulating bilirubin derived from the injected hemoglobin was about 100 mgm. However, as will be seen from Figures 1 and 3, the urinary excretion of coproporphyrin remained at a level of approximately 500 micrograms per 24 hours.

It is of interest that, in this test, the injection was followed by the excretion of 0.47 mgm. of hemoglobin in the urine, and in the 24 hours following the injection 7 mgm. of urobilinogen appeared in the urine.

As a control to these experiments, the levels of plasma bilirubin and urinary coproporphyrin excretion were measured over a previous 24-hour period. As may be seen in Figure 4, their levels did not fluctuate markedly.

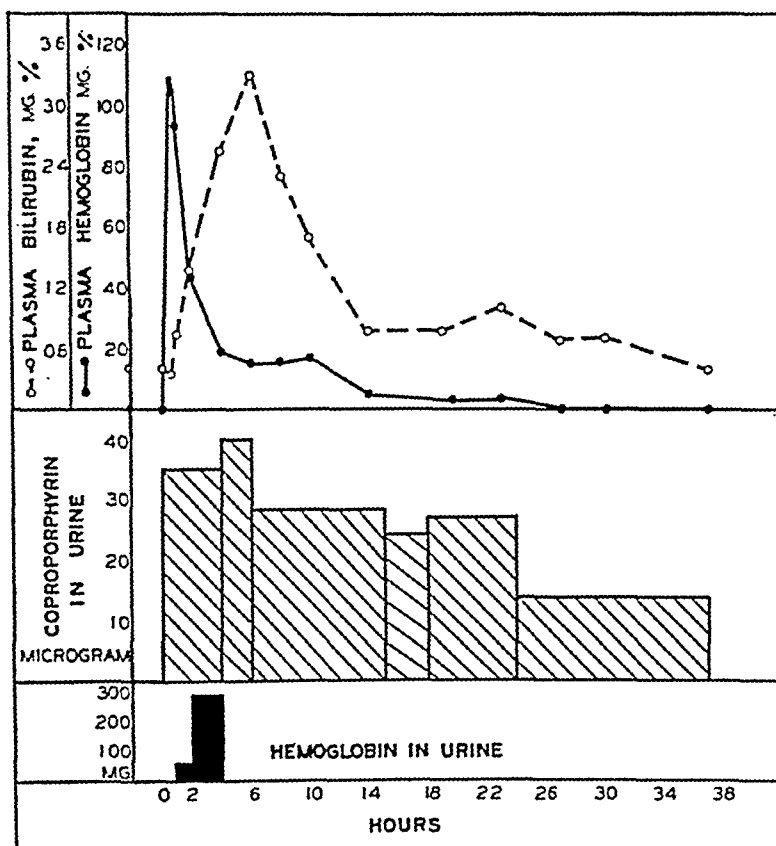


FIG. 3. CASE 1. PLASMA BILIRUBIN AND HEMOGLOBIN AND URINARY COPROPORPHYRIN AND HEMOGLOBIN FOLLOWING THE INTRAVENOUS ADMINISTRATION OF ABOUT 19 GRAMS OF HEMOGLOBIN

Case 2. About 7.9 grams of hemoglobin derived from 100 ml. of the patient's own blood were injected. The amount of protoporphyrin which would be liberated if all this hemoglobin were broken down is about 260 mgm. Following the injection, the plasma bilirubin showed a maximum increase of 1.1 mgm. per cent (Figure 5). The red blood cell volume was 38 per cent and, assuming again that the blood volume was approximately 5 liters, 33 mgm. of bilirubin derived from the injected hemoglobin may be assumed to have been circulating in the blood stream at this time. The urinary excretion of coproporphyrin during the test is recorded in Figures 2 and 5. It will be seen that no variations, even of the order of 100 micrograms, were observable in the 24-hour amount excreted.

There was no significant change in the fecal excretion of urobilinogen or coproporphyrin (Figure 4), though 0.8 mgm. of hemoglobin appeared in

the urine shortly after the injection and 14 mgm. of urobilinogen was excreted in the urine during the succeeding 24 hours.

DISCUSSION

In both these patients the introduction of free hemoglobin into the blood stream was followed by a rapid rise in plasma bilirubin. This rise in bilirubin resembles very closely both in degree and time of onset the bilirubinemia observed by Gilligan *et al.* (11) in normal subjects under the same conditions. It is evident, therefore, that these two patients were able to convert hemoglobin to bilirubin without evidence of impairment of this process. Nevertheless, the transient increase in hemoglobin destruction produced by this experimental means resulted in no appreciable alteration in the amount of coproporphyrin excreted in the urine or feces, although the amount of hemoglobin injected was presumably sufficient to have pro-

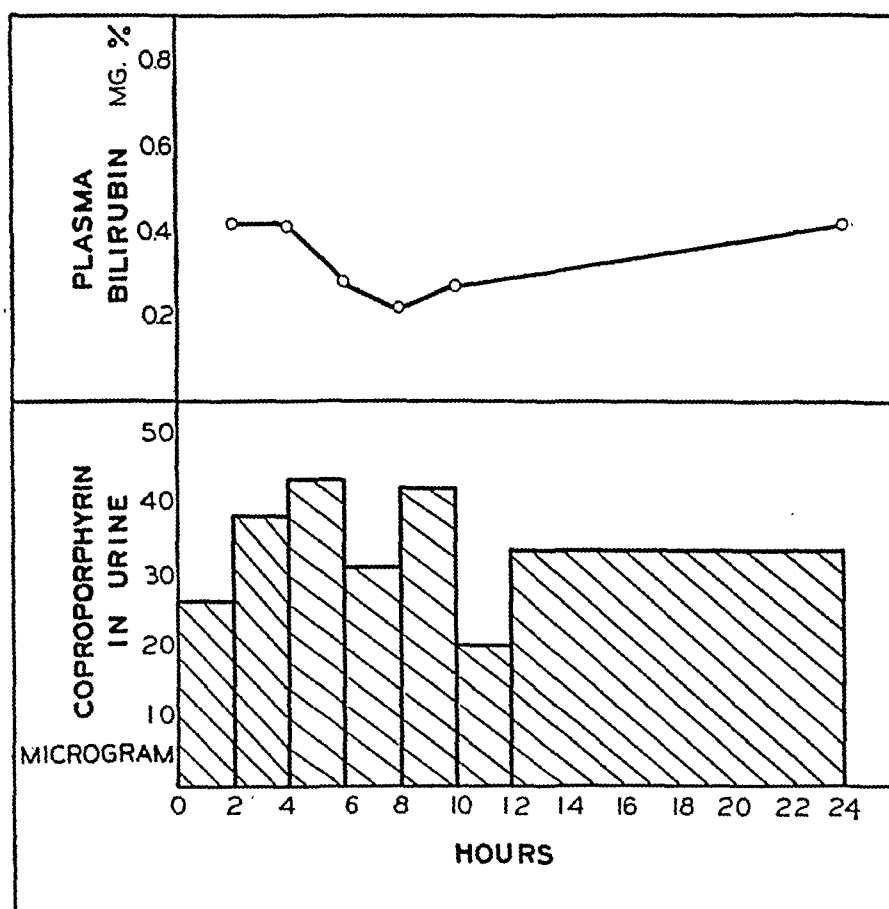


FIG. 4. CASE 1. PLASMA BILIRUBIN AND URINARY COPROPORPHYRIN DURING A 24-HOUR CONTROL PERIOD

duced a definite rise in porphyrin excretion if any significant part of it had been eliminated in this form. These results suggest that there is no obvious defect in the mechanism of hemoglobin destruction in lead poisoning, and consequently that the porphyrinuria characteristic of this condition cannot be explained on the basis of such a defect occurring as a result of increased destruction of red blood cells.

A possible objection to this conclusion is the fact that these experiments did not succeed in demonstrating the ultimate fate of the injected hemoglobin. Though it was not excreted as porphyrin, it did not appear as an increase in fecal urobilinogen as might have been expected. However, it seems certain that at least some part of the hemoglobin was broken down, as judged by the consistent appearance of bilirubin in the plasma after each injection of hemoglobin, and the transient increase in urobilinogen in the urine in two instances. If a defect in hemoglobin breakdown was responsible for the increased porphyrin excretion before the injection of hemoglobin, one

would have expected that at least a few additional milligrams of porphyrin would have appeared in the urine or feces as a result of the hemoglobin injections. However, no detectable alteration in porphyrin excretion was observed. The lack of increase in the fecal excretion of urobilinogen following the injection of hemoglobin was probably due to the conservation of bilirubin within the body. A similar discrepancy between the amount of hemoglobin destroyed and the excretion of its waste products in the bile has been noted by other authors (12).

It is logical to conclude that in plumbism the porphyrin excreted in the urine is not derived from any abnormal destruction of red blood cells, but is more probably a result of defective synthesis of hemoglobin. This conclusion is in agreement with the views recently expressed by Watson (13) and by Rimington (14). Watson states "It seems more likely that the formation of coproporphyrin III is related to a disturbance in the formation of hemoglobin rather than to its destruction." Rimington believes that the abnormal excretion of

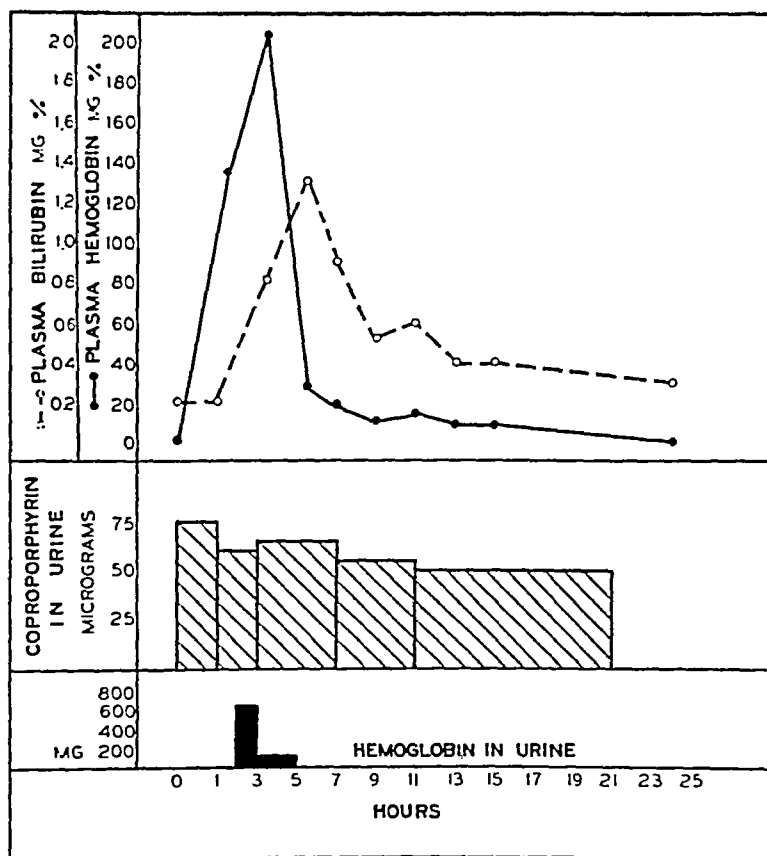


FIG. 5. CASE 2. PLASMA BILIRUBIN AND URINARY EXCRETION OF COPROPORPHYRIN AND HEMOGLOBIN FOLLOWING THE INTRAVENOUS ADMINISTRATION OF ABOUT 7.9 GRAMS OF HEMOGLOBIN

coproporphyrin III results from partial block by toxic substances of the entrance of iron into the porphyrin ring and consequent defective synthesis of hemoglobin.

In view of this evidence it seems pertinent to reconsider the traditional belief that the anemia of lead poisoning is hemolytic in nature, and to inquire whether there is a possibility that defective hemoglobin synthesis may play a part in its production. The traditional hypothesis is supported by the following evidence. Aub, Reznikoff and Smith (15) clearly showed that if lead salts are added to normal blood the red cells shrink and seem to become more brittle, with resulting "fractionation" and escape of hemoglobin. Key (16) has produced a marked anemia in rabbits following the administration of lead salts, resulting in the loss of over one million red blood cells per cubic millimeter in 24 hours. Furthermore, the anemia of lead poisoning in man is accompanied by an

apparent reticulocytosis comparable to the reticulocytosis occurring in hemolytic jaundice; it is possible, however, that this reticulocytosis may be of a "spurious" type such as occurs in cases of pernicious anemia treated with arsenic.

Against the hemolytic hypothesis it may be said that, although Aub and his collaborators showed that hemolysis may be caused by the addition of lead salts to blood *in vitro*, the amount of lead employed to produce this effect was very much more than is usually present in the blood in chronic lead poisoning. Thus, although their results may well explain the acute anemia produced in rabbits by Key, it is doubtful whether they furnish an explanation of the anemia in chronic lead poisoning.

As Watson (13) has pointed out, the anemia in human lead poisoning is usually associated with a color index below 1.0, and by a reduced mean corpuscular hemoglobin concentration. This sug-

gests a disturbance or retardation in the formation of hemoglobin. Moreover, as he (17) has shown, the excretion of urobilinogen in the feces is not increased as in other hemolytic conditions. This observation is confirmed by the results reported in the present communication. It must be concluded that, while the anemia produced by acute lead poisoning in rabbits is apparently hemolytic in nature, the evidence is against the view that the anemia commonly observed in chronic human lead poisoning can be explained on the same basis.

It is pertinent now to consider the possible origin of the coproporphyrin found in the urine in lead poisoning. Watson and Clarke (18) have lately shown that reticulocytes contain a considerable amount of protoporphyrin, and have suggested that this protoporphyrin is the material responsible for the reticulum in these cells. It is tempting to suggest that the characteristic appearance of the stippled cells found in the blood in lead poisoning is also due to a porphyrin, though of sufficiently different composition to modify its morphologic appearance. As pointed out above, there is evidence that the synthesis of hemoglobin is impaired in lead poisoning. The appearance of stippling in the red cells may therefore be an expression of an arrest in the maturation of hemoglobin and consequent accumulation of porphyrin products within the cells. It seems likely that, when the stippled cells are ultimately destroyed, this material would be liberated and, being an abnormal metabolite, might be excreted in an abnormal manner. This, therefore, might well account for at least some part of the larger amount of coproporphyrin III appearing in the urine. In support of this view, the observations of Thomas (19) may be mentioned. He has pointed out that, whereas hemoglobin injected into animals is excreted as bile pigments, injected hematin is recovered as porphyrin in the urine.

Whatever the ultimate explanation of porphyrinuria in lead poisoning, we believe that it is at least very intimately related to the same metabolic disturbance that gives rise to the stippling in the red blood corpuscles. This belief is borne out by some unpublished observations in which we found that, in a group of fourteen lead workers with porphyrinuria, the majority also showed the presence of stippled cells in the blood.

SUMMARY AND CONCLUSIONS

Solutions of hemoglobin were injected into two subjects with lead poisoning, anemia and porphyrinuria. The injection was followed by a rise in plasma bilirubin resembling closely both in degree and time of onset the bilirubinemia which has been observed (11) in normal subjects under comparable conditions. It was also accompanied by transient increase in urinary urobilinogen excretion. The injection resulted in no detectable increase in the excretion of coproporphyrin either in the urine or feces.

These results failed to demonstrate any interruption in the path by which hemoglobin is destroyed in the body. It is, therefore, concluded that the porphyrinuria occurring in lead poisoning cannot be explained on this basis.

The relation of these findings to the problem of the causation of anemia in lead poisoning is discussed. It is concluded that they lend support to the view that this anemia is dyshematopoietic rather than hemolytic in nature.

We wish to express our grateful thanks to Miss Constance Brooks who performed the bilirubin and hemoglobin estimations, and to Frank P. Cohen, M.S., for his valuable assistance in carrying out quantitative determinations of porphyrin and urobilinogen. We also wish to thank Dr. Dorothy Rourke Gilligan for her advice on the procedure for injecting hemoglobin.

BIBLIOGRAPHY

1. Garrod, A. E., The occurrence and detection of hematoporphyrin in the urine. *J. Physiol.*, 1892, 13, 598.
2. Grotepass, W., Zur Kenntnis des in Harn auftretenden Porphyrins bei Bleivergiftung. *Ztschr. f. physiol. Chem.*, 1932, 205, 193.
3. Watson, C. J., Concerning the naturally occurring porphyrins. IV. The urinary porphyrins in lead poisoning as contrasted with that associated normally and in other diseases. *J. Clin. Invest.*, 1936, 15, 327.
4. Brugsch, J. T., and Keys, A., Quantitative separation and estimation of various porphyrins in biological materials. *Proc. Soc. Exp. Biol. and Med.*, 1938, 38, 557.
5. Kark, R., and Meiklejohn, A. P., Pellagra and porphyrinuria. *Am. J. M. Sc.*, 1931, 201, 380.
6. Watson, C. J., Studies of urobilinogen. I. An improved method for the quantitative estimation of urobilinogen in urine and feces. *Am. J. Clin. Path.*, 1936, 5, 458.

7. Bing, F. C., and Baker, R. W., The determination of hemoglobin in minute amounts of blood by Wu's method. *J. Biol. Chem.*, 1931, 92, 589.
8. Ottenberg, R., and Fox, C. L., The rate of removal of hemoglobin from the circulation and its renal threshold in human beings. *Am. J. Physiol.*, 1938, 123, 516.
9. Barron, E. S. G., Bilirubinemia. *Medicine*, 1931, 10, 77.
10. Gross, E. S., Sasaki, Y., and Spies, T. D., Effect of nicotinic acid on increased porphyrinuria occurring in seven painters. *Proc. Soc. Exp. Biol. and Med.*, 1938, 38, 289.
11. Gilligan, D. R., Altschule, M. D., and Katevsby, E. M., Studies of hemoglobinemia and hemoglobinuria produced in man by intravenous injection of hemoglobin solution. *J. Clin. Invest.*, 1941, 20, 177.
12. Watson, C. J., in Downey's Handbook of Hematology. Paul B. Hoeber, Inc., New York, 1938, vol. IV, p. 2490.
13. Watson, C. J., in a symposium on the blood and blood forming organs. University of Wisconsin Press, Madison, 1939, p. 25.
14. Rimington, C., Porphyrinuria following sulphanilamide: Sulphanilamide dermatitis. *Lancet*, 1938, 1, 770.
15. Aub, J. C., Reznikoff, P., and Smith, D. E., Lead studies. III. The effect of lead on red blood cells. Part I. Changes in hemolysis. *J. Exper. Med.*, 1924, 40, 151.
16. Key, J. A., Lead studies. IV. Blood changes in lead poisoning in rabbits, with especial reference to stippled cells. *Am. J. Physiol.*, 1924, 70, 86.
17. Watson, C. J., Concerning the naturally occurring porphyrins. V. Porphyrins of the feces. *J. Clin. Invest.*, 1937, 16, 383.
18. Watson, C. J., and Clarke, W., The occurrence of protoporphyrin in the reticulocytes. *Proc. Soc. Exp. Biol. and Med.*, 1937, 36, 65.
19. Thomas, J., Contribution à l'étude des porphyrines en biologie et en pathologie. Duclume. Lonx-le-Saunier, 1938, p. 73, 121.

THE ACID-BASE BALANCE OF PREMATURE INFANTS¹

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The acid-base balance of young children is known to be easily upset (1, 2) and that of full-term new-born infants changes with slight variations in diet (3, 4). Premature infants are considered to be even more labile, but data are few. The symptoms of acidosis in prematures, such as hyperpnea, etc., may resemble those of older children, but often the condition may not be recognized clinically because sluggishness and refusal of feedings are the only signs. However, only those displaying hyperpnea were classified as acidotic for purposes of comparison in this paper. In order to obtain more data and to investigate the cause of their frequent acidosis, the acid-base balance was studied in seventeen premature infants whose birth weight was less than 2250 grams. Clinical aspects of these cases are discussed elsewhere (5).

MATERIAL AND METHODS

Alternate patients were fed dilute Olac or equal parts of evaporated milk and water with the addition of 7½ per cent cane sugar in amounts supplying 120 calories per kilo per day. All patients received 25 mgm. ascorbic acid daily after the thirteenth day, and 10 drops of oleum percomorphum daily after the twentieth day of life. Patients with clinical signs of acidosis were treated with M/6 sodium lactate parenterally, 60 cc. per kilo body weight.

Blood was drawn under oil with heparin as anticoagulant. Single specimens of urine were taken in test tubes; twenty-four-hour specimens were collected from males to whom funnels were strapped with tubing extending to urine receptacles containing toluene. In normal infants blood was drawn halfway through the twenty-four-hour urine collection.

Total base and chlorides of urine and blood were determined by electrolysis (6). Formol titration of urinary cation mixture measured the ammonia. These results were checked by the method of Folin and Bell (6b).

Blood pH was measured in glass micro-electrodes, and the results were corrected by +0.05 pH to compensate

for the immediate acid change (7). Urine pH was measured with glass macro-electrodes.

Carbon dioxide content of plasma was determined in the Van Slyke manometric apparatus (8). Phosphorus in the blood was determined by the method of Kuttner and Lichtenstein (9), and in urine by that of Youngburg and Youngburg (10). Plasma proteins were determined by the direct nesslerization method of Wong (11) and read in the Evelyn photoelectric colorimeter.

Total organic acids in plasma were calculated by subtraction of the total anion content from the total base, expressed as milliequivalents per liter. For this purpose, sulfates were assumed to account for two milliequivalents per liter. Total organic acids in urine were determined by the method of Greenwald (12) adapted for titration with the glass electrode. Lactic acid in blood was determined by the method of Elgart and Harris (13), and in urine by that of Friedemann, Cotonio and Shaffer (14).

RESULTS AND DISCUSSION

Table I lists the findings on the blood of fifteen premature infants when all clinical signs indicated

TABLE I
Premature infants in good condition
Determinations on blood plasma in m.eq. per liter

Patient	Birth weight	Age	pH	Total base	CO ₂ content	Cl	PO ₄	Base-binding power of protein	Organic acid† (calculated)
Number	Sex	grams	days						
A44733	F	1275	58	160	14.5	102	3.6	(12.4)*	25.5
A45677	M	1905	40	153	18.2	106	3.6	(12.4)	10.8
A46111	M	1960	30	155	20.5	108	3.8	(12.4)	8.3
A46353	M	1675	20	160	15.3	103	4.9	(12.4)	22.4
		50		151	18.3	101	4.5	(12.4)	12.8
A46383	M	1725	42	160	16.6	104	3.8	(12.4)	21.2
A48228	M	1420	18	148	18.7	105	4.0	(12.4)	6.0
		42	7.28	163	16.0	104	3.3	(12.4)	23.0
A49834	M	1945	16	159	13.8	106	4.2	(12.4)	20.6
		34	7.42	151	16.0	104	4.2	11.8	13.0
A49793	M	1700	22	132	13.3				
		39	7.36	160	14.1	103	3.9	12.4	24.6
A50429	M	2010	16	151	13.6	104	3.5	(12.4)	14.0
		26	7.33	149	15.9	101	5.2	12.0	12.9
A53370	M	1700	3	160	22.3	104	3.9	14.1	13.7
A53371	M	1540	3	154	14.0	100	4.4	13.3	20.3
A53019	M	1950	11	132	16.0	103	4.3	10.5	16.2
A52808	M	2200	23	155	21.0	104	4.4	12.1	21.5
A52454	M	2235	19	156	16.0	105	4.8	10.3	17.9
A52940	F	1300	36	163	24.7	97	3.5	13.6	22.8
Average			7.33	156	16.8	103	4.1	12.4	17.8

* Figures in parentheses are averages discussed in text.
† SO₄ is assumed to be 2 m.eq. per liter of plasma for calculation of organic acid content.

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a satisfactory condition. All of them had total base and chlorides within the limits of normal. Plasma phosphates were high, as is usual in infants. Prematures with clinical evidence of acidosis (hyperpnea, etc.) were also normal in these respects (Table II).

TABLE II
Clinically acidotic prematures

Determinations on blood plasma in m. eq. per liter

Patient		Age	pH	Total base	CO ₂ content	Cl	PO ₄	Base-binding power of protein	Organic acid (calculated)*
Number	Sex								
		<i>days</i>							
A44733	F	62		150	9	99	4.6	(12.4)†	23
A46353	M	44			11			(12.4)	
A46383	M	37		154	13.5	96	3.7	(12.4)	28
A52808	M	12	7.31	155	16	103	4.2	12.9	16.5
A52454	M	14	7.26	149	11	107	4.8	12.6	11.6
A45227	M	13		160	10	108	5.0	(12.4)	22.6
A52425	F	45	7.24	160	14.1	103	3.5	13.6	23.7

* SO₄ is again assumed to be 2 m.eq. per liter.

† Figures in parentheses are averages discussed in text.

The carbon dioxide content of the plasma was uniformly low. Patient A52940, who had a carbon dioxide content of 24.7 milliequivalents per liter, had received 4.0 cc. of 1.0 molar sodium lactate orally every eight hours for four days preceding the determinations. The blood pH roughly parallels the carbon dioxide content. Hyperpneic prematures had slightly lower levels, but the distinction was not sharp. The highest carbon dioxide content of the acidotic group, 16.0 milliequivalents per liter, was well above the lowest in the thriving group, yet the latter progressed satisfactorily without respiratory or feeding irregularity, and without prophylaxis against acidosis.

The plasma proteins of the last nine infants in the normal series were determined simultaneously with the other tests. For the first six, a figure of 12.4 milliequivalents per liter was assumed, this being the average value from more than thirty determinations on the later infants, and on other prematures not in the series. Patients A46111 and A48228 were slightly edematous at the time of sampling, so that this figure is probably too high in their cases. There was no remarkable difference between the well and acidotic infants on this score.

The calculated organic acid contents of the plasma indicated an amount two to four times the

amount considered normal for older patients. They varied inversely as the carbon dioxide content, and were only slightly higher in the acidotic than in the clinically well infants. Figure 1 is il-

TABLE III
Premature infants in good condition
Determinations on urine in m.eq. per liter

Patient	Age	pH	Total base	NH ₃	Cl	Phosphorus	Total organic acids	Urinary lactic acid	Undetermined organic acids	Volume	M.eq. organic acid per kilo body weight per day
	<i>days</i>					<i>grams per liter</i>				<i>cc. per 24 hours</i>	
A46353	50				54.0	1.50				137	
A46383	42	7.2			19.6	1.80				62	
A48228	18	5.46			48.6	0.77				132	
	42	5.40	126.0		48.2	1.08				160	
A49834	18	5.10	138.4		46.8	0.67				160	
	34	5.02	64.0	29.0	35.0	1.17	78.0			155	4.48
A49793	22	5.24	186.7		70.4	1.46				110	
	39	4.96	100.0	39.2	38.0	1.91	105.0			126	5.29
A50429	16	5.31	137.0		29.6	0.95				120	
	26	4.90	66.2	18.8	40.0	1.43	62.0			110	3.14
A53370	3	5.15	86.5	17.0	52.7	0.33	20.0	1.3	18.7	126	1.48
A53371	3	5.18	154.6	35.4	80.0	0.85	30.0	8.8	21.2	83	1.56
A53019	11	5.15	165.0	51.0	85.0	1.60	59.4	11.3	48.1	68	1.96
A52808	23	6.13	138.4	16.1	57.8	0.77	20.0	2.4	17.6	210	1.38
A52454	19	5.48	129.0	37.2	23.7	1.19	33.0	1.02	31.98	180	1.86
A52940	36	5.52	173.0	81.0	34.0	0.99	83.0				

TABLE IV
Clinically acidotic prematures
Determinations on urine in m.eq. per liter

Patient	Age	pH	Total base	NH ₃	Cl	Phosphorus	Organic acids	Lactic acid
	<i>days</i>					<i>grams per liter</i>		
A44733*	62	5.61			51	0.48		
After 120 cc. 1/6 M Na Lactate		5.52			86	1.15		
A46353	44				46	0.62		
After 105 cc. 1/6 M Na Lactate					53	1.10		
A46383	37	5.28			26	0.70		
After 120 cc. 1/6 M Na Lactate		4.89			29	0.91		
A52808	12	5.30	90	37	58	0.70	22.0	2.1
After 75 cc. 1/6 M Na Lactate		4.94	184	86	44	2.00	50.0	5.4
A52454	14	5.20	135	34	62	1.23	32	11.0
After 140 cc. 1/6 M Na Lactate		4.90	185	59	39	1.14	97	72.0
A45227	13	5.6			34	0.98		
A52425	45	5.32	221.7	107	64.2	2.50	100.0	
After 60 cc. 1/6 M Na Lactate		4.89	232.6	122	38.4	2.56	116.0	

* Died three days after this determination. This was the only infant in the series who did not recover promptly.

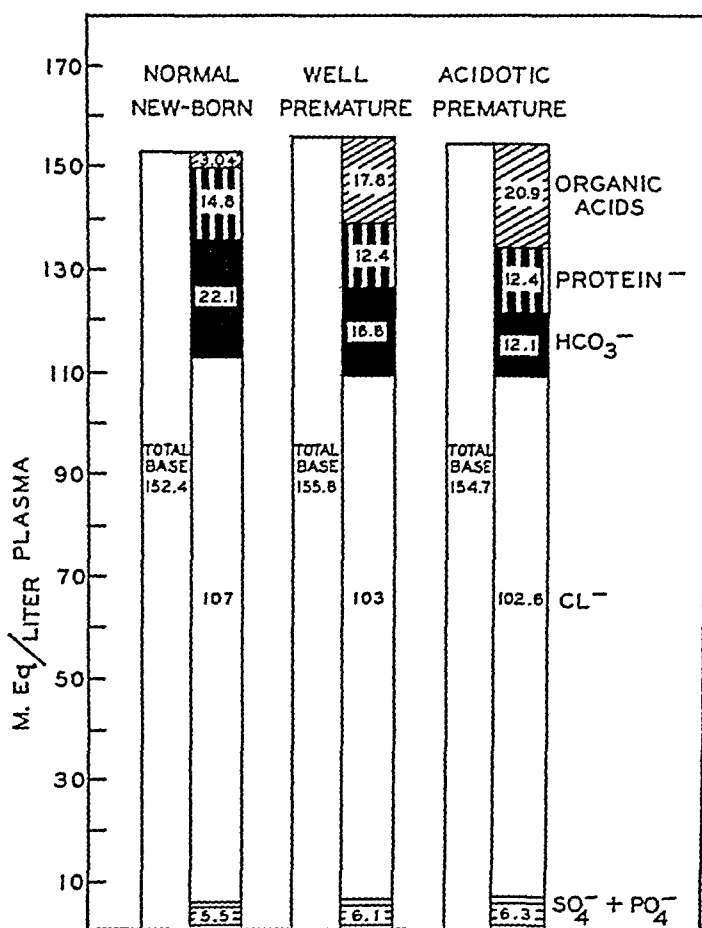


FIG. 1. DIAGRAM COMPARES AVERAGE FIGURES FOR TOTAL BASE AND VARIOUS ANIONS OF PLASMA OF NORMAL NEW-BORN INFANTS, 15 PREMATURES WHO WERE WELL CLINICALLY, AND 6 PREMATURES IN ACIDOSIS (HYPERPNEIC)

Figures for normal new-born infants were taken from Marples and Lippard (3) whose organic acid estimation is probably low.

lustrative of the anion relations in the blood of full-term new-born infants in comparison with well and acidotic prematures.

All twenty-four-hour urine specimens were remarkably acid in spite of unavoidable loss of carbonic acid (Table III). Total base, ammonia, chloride and phosphorus followed no pattern, but it is worthy of notice that less fixed base is replaced by ammonia than might be expected in such acid urine.

The high calculated values for plasma organic acids are supported by the excretion of two to five times the normal adult amount per kilo body weight (12, 15). Added interest is warranted by

the absence of the usual ketone bodies from the blood and urine of every infant studied.

When discovered in hyperpnea, an infant was strapped for a single specimen, and blood for determinations was drawn. As soon as the patient voided, M/6 sodium lactate was given and the patient was strapped for a second specimen. The urine of the prematures in clinical acidosis (Table IV) was not as acid as expected, and it is remarkable that, after receiving the sodium lactate, the urine became more acid than before. Likewise, ammonia excretion increased proportionately, and the amount of phosphates was doubled. Because lactate was used therapeutically, lactic acid

excretion was measured on some of the normal and acidotic infants. In the normal infants, it was usually low, and amounted at most to 20 per cent of the urinary organic acids in one case. Twenty normal prematures were found to have blood lactic acids varying from 2.7 to 4.2 milliequivalents per liter, with an average of 3.5 milliequivalents per liter. The increased excretion of organic acids after therapy is accounted for largely, though not entirely, by lactate. Sodium salts of certain organic acids received by infants are known to be excreted unchanged (16, 17). Such is probably the case here, but since the carbon dioxide content of plasma of these infants rises, and since weight gain is accelerated for the few days following therapy, a portion of the dose may be considered metabolized and its cations used to balance other anions for storage or excretion.

While this study was in progress, work was published on the presence of abnormal products of the metabolism of tyrosine and phenylalanine in the urine of premature infants who had received no ascorbic acid (18, 19). All the patients except A53370, A53371, and A53019 had been given 25 mgm. of ascorbic acid daily before determinations were done, beginning on the fourteenth day of life. Determinations were done on the urine of the last four patients, and on three other prematures not in this series, but no excretion of total hydroxy phenyl compounds was found to be greater than 64 mgm. per twenty-four hours, expressed as tyrosine (20).

The low plasma proteins found in these infants are probably part of the inefficiency evidenced in prematures by their well-known iron deficiency and their difficulty in the production of hemoglobin. In this connection, the excretion of less acid urines during acidosis than after therapy with fixed base brings into question the sufficiency of the premature kidney. Temporary functional renal insufficiency has been reported in cases of diabetic acidosis (21) in which azotemia associated with reduced renal clearance was rectified by restoration of normal acid-base and electrolyte balances. In premature infants the renal insufficiency is probably functional as well as due to immaturity, since after therapy they excrete not only a more acid urine, but a higher proportion of ammonia for the neutralization of urinary acids.

Urea clearance and blood nonprotein nitrogen studies would help clear up this point.

The addition of small amounts of fixed base to their feedings has been shown to benefit full-term new-born infants (4, 22). This is true also of prematures, but an increased supply of base seems only to facilitate the work of the kidney, not to correct the metabolic defect. At present an investigation is in progress in this laboratory to single out the particular organic acids involved. It is hoped that this study may indicate whether the entire metabolism of the premature is inefficient or whether some particular oxidative mechanism is at fault.

SUMMARY AND CONCLUSIONS

1. The acid-base balance of seventeen premature infants has been studied.
2. The plasma content of organic acids of premature infants is two to three times as great as that of the full-term infant and the adult. Ketone bodies are not responsible for this excess of organic acids.
3. Premature infants excrete two to five times the normal adult amount of organic acid per kilo body weight per day.
4. Although the electrolyte pattern in the plasma differs greatly from that of the term infant, it is fairly rigidly maintained and is not appreciably altered by alkali therapy.
5. The organic acid and carbon dioxide contents of plasma of clinically acidotic (hyperpneic) premature infants differ little from those of infants who are clinically thriving. This is taken to indicate that the premature infant is always on the borderline of acidosis.

BIBLIOGRAPHY

1. Gamble, J. L., Ross, G. S., and Tisdall, F. F., Studies of tetany; the effect of calcium chloride on the acid-base metabolism of infants. *Am. J. Dis. Child.*, 1923, 25, 455.
2. Wilson, J. R., Levine, S. Z., and Rivkin, H., The respiratory metabolism in infancy and childhood; ketosis and the respiratory exchange in children. *Am. J. Dis. Child.*, 1926, 31, 335.
3. Marples, E., and Lippard, V. W., Acid-base balance of new-born infants; influence of cow's milk on the acid base balance of the blood of new-born infants. *Am. J. Dis. Child.*, 1933, 45, 294.

4. Lippard, V. W., and Marples, E., Acid-base balance of new-born infants; effect of ingestion of alkali on acid-base balance of new-born infants. *Am. J. Dis. Child.*, 1933, 46, 495.
5. McBryde, A., and Branning, W. S., Acidosis in premature infants; a clinical report. (In preparation.)
- 6a. Joseph, N. R., and Stadie, W. C., The simultaneous determination of total base and chloride on the same sample of serum by electrodialysis. *J. Biol. Chem.*, 1938, 125, 795.
- b. Folin, O., and Bell, R. D., Applications of a new reagent for the separation of ammonia; the colorimetric determination of ammonia in urine. *J. Biol. Chem.*, 1910-11, 8, 497.
7. Havard, R. E., and Kerridge, P. T., An immediate acid change in shed blood. *Biochem. J.*, 1929, 23, 600.
8. Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Vol. II. Methods, pp. 282-288, Williams and Wilkins Company, Baltimore, 1932.
9. Kuttner, T., and Lichtenstein, L., Micro-colorimetric studies; estimation of phosphorus: molybdcic acid-stannous chloride reagent. *J. Biol. Chem.*, 1930, 86, 671.
10. Hawk, P. B., and Bergeim, O., *Practical Physiological Chemistry*, Tenth Edition, pp. 875-876, P. Blakiston's Son and Co. Philadelphia, 1931.
11. Wong, S. Y., The use of persulfate in the estimation of nitrogen by Folin's direct nesslerization method. *J. Biol. Chem.*, 1923, 55, 431.
12. Greenwald, I., Studies on metabolism in pneumonia; the excretion of "organic acid" and a method for its determination. *J. Biol. Chem.*, 1930, 85, 447.
13. Elgart, S., and Harris, J. S., The determination of lactic acid in blood. *Anal. E. Ind. Eng. Chem.*, 1940, 12, 758.
14. Friedemann, T. E., Cotonio, M., and Shaffer, P. A., The determination of lactac acid. *J. Biol. Chem.*, 1927, 73, 335.
15. Van Slyke, D. D., and Palmer, W. W., Studies of acidosis; the titration of organic acids in urine. *J. Biol. Chem.*, 1920, 41, 567.
16. Smith, A. H., and Orton, J. M., Editorial review. The nutritional and metabolic significance of certain organic acids. *J. Nutrition*, 1937, 13, 601.
17. Smith, A. H., Barnes, D. J., Meyer, C. E., and Kaucher, M., Metabolism of citric acid by infants. *J. Nutrition*, 1940, 20, 255.
18. Levine, S. Z., Marples, E., and Gordon, H. H., A defect in the metabolism of aromatic amino acids in premature infants. *Science*, 1939, 90, 620.
19. Levine, S. Z., Gordon, H. H., and Marples, E., A defect in the metabolism of tyrosine and phenylalanine in premature infants; spontaneous occurrence and eradication by vitamin C. *J. Clin. Invest.*, 1941, 20, 209.
20. Medes, G., A new error of tyrosine metabolism: Tyrosinosis. The intermediary metabolism of tyrosine and phenylalanine. *Biochem. J.*, 1932, 26, 917.
21. McCance, R. A., and Widdowson, E. M., Functional disorganization of the kidney in disease. *J. Physiol.*, 1939, 95, 36.
22. Marples, E., and Lippard, V. W., Acid-base balance of new-born infants; consideration of the low alkaline reserve of normal new-born infants. *Am. J. Dis. Child.*, 1932, 44, 31.

RENAL FUNCTION IN PATIENTS WITH ADDISON'S DISEASE AND IN PATIENTS WITH ADRENAL INSUFFICIENCY SECONDARY TO PITUITARY PAN-HYPOFUNCTION¹

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Acute adrenal insufficiency is a complex phenomenon and at least three dysfunctions of body economy may accompany its development in humans. The disturbances which are intimately associated with the pathogenesis of adrenal insufficiency in patients suffering from Addison's disease are: (a) dissipation of water and sodium with retention of potassium, (b) impaired glycogenesis from protein, and (c) depression of renal activity. The existence of these disorders has been assumed from the clinical findings of hyponatremia and hyperproteinemia, hypoglycemia, and azotemia, respectively. Experimental studies concerning the precise dependence or the interdependence of these disturbances are not yet conclusive. Whether or not the several dysfunctions are interdependent, they are partially susceptible to investigation, independently. Thus it is possible to correct temporarily the disturbance of electrolyte exchange, meanwhile exerting little influence upon impaired glycogenesis. A clinical example of such an independence (1) is seen in patients with Addison's disease who die in hypoglycemic shock with a normal concentration of serum sodium.

Experimental studies recently performed suggest that impairment of renal function also may be independent of alteration of glycogenesis as well as independent of the typical disturbances of electrolyte equilibrium. The hypothesis has been tested in humans by quantitative measurements of the various functions of the kidney at different levels of adrenal activity. The results will be presented in this communication.

There are few studies reported in the literature which are pertinent to the discussion. The first reports were published in 1914 when three observers, independently, discussed the development of renal insufficiency in association with acute adrenal cortical insufficiency. Sicard and Haguénau (2) dismissed the matter with the statement that kidney insufficiency may be observed in humans during an adrenal crisis. Gaillard (3) made the first quantitative measurements of renal insufficiency and noted a blood urea of 320 mgm. per 100 cc. in a patient who had died from adrenal failure. A similar increase of waste products in the blood was noted by Porak and Chabanier (4) in animals following bilateral adrenalectomy. In 1916 Marshall and Davis (5) observed a delayed excretion of phenolsulphonphthalein dye as well as nitrogen retention in the adrenalectomized dog. They ascribed the changes to a "functional depression of the kidneys caused by a failure of elaboration by the adrenals of a substance necessary for maintenance of normal kidney function." Renal function in chronic adrenal insufficiency was measured by Stahl, Atchley and Loeb (6) in an adrenalectomized dog which was maintained for 9 months with adrenal cortical extract and salt without evidence of a progressive decrease in phenolsulphonphthalein excretion or urea clearance. A diminution in renal function was demonstrated only during an adrenal crisis which could be induced by withdrawal of either salt or extract. It is possible that if the dog had suffered from insidious chronic adrenal insufficiency for as long a time as do many patients with Addison's disease, evidence of renal damage might have been evident at other times than during an adrenal crisis.

Investigation of renal function during intercritical periods in a series of patients with Addison's disease was pursued first by Rowntree (7) who reported a decreased excretion of phenolsulphonphthalein dye 2 hours after injection in ten out of twelve patients. It is regrettable that fractional collection of urine samples was not practiced since the amount of dye excreted during a 2-hour collection period is not as accurate an index of mild renal insufficiency as is the per cent excreted in the first 15 minutes after injection. Subsequently, Rowntree and Snell (8) noted a decreased excretion of dye in acute adrenal insufficiency as well as hyposthenuria, albuminuria and cylinduria in patients with Addison's disease whose symptoms were controlled. Inability to concentrate urine was observed by Rosenow (9) in two patients with

¹This investigation was aided by the Corn Industries Research Foundation. The authors wish to thank Dr. George W. Thorn of Baltimore and the Ciba Pharmaceutical Products Company for the generous quantities of desoxycorticosterone acetate (Percorten) supplied in oil and in pellets.

Addison's disease. He concluded that ability to concentrate urine was a function of the endocrine glands.

It is agreed that albuminuria, cylinduria and hypos-thenuria are indicative of grave insults to the kidney. Less devastating alterations might occur, but would escape recognition because accurate measurements of small variations from the normal are not possible with the phenol-sulphonphthalein and urine concentration tests. On the other hand, dependable quantitative methods for determination of renal efficiency by measuring rate of clearance of creatinine, inulin, diodrast and glucose have been developed only recently and have not been applied generally to patients with Addison's disease. Particularly pertinent is the application of these tests during the inter-critical periods when symptoms are adequately or almost completely controlled as the result of administration of sodium chloride and hormones.

The creatinine clearance test devised by Rehberg (10) which measures, in humans, tubular excretory function as well as rate of glomerular filtration was applied by Margitay-Becht and Gömöri (11) to three patients with Addison's disease. Values of 68, 63, and 56 cc. of plasma cleared per minute, respectively, were observed during crises. Following parenteral administration of adrenal cortical extract, the rates increased to 105, 109, and 144. All of these values are below normal except the last. It is stated that the depression in creatinine clearance showed no direct correlation with alteration of blood pressure, serum nonprotein nitrogen, serum sodium or colloid osmotic pressure of the serum. It is thought that these are the first data which show incomplete restoration of impaired tubular function and glomerular filtration in patients with Addison's disease whose symptoms are controlled by administration of adrenal cortical extract. McCance (12) reported an isolated datum of 50 cc. of glomerular filtrate formed per minute during an inulin clearance test in one patient with Addison's disease. No statement was made regarding symptoms, but the patient presumably was not in a severe crisis since he concluded that while hypotension, dehydration, diminution in plasma volume, hyperproteinemia, and uncompensated alkalosis might contribute to the pathogenesis of renal dysfunction in acute renal insufficiency, these factors would not explain the diminution in kidney efficiency observed. Finally, Gersh and Grollman (13) investigated glomerular filtration and tubular reabsorption of ferrocyanide salts in the rabbit, the dog, and the cat. These functions were studied immediately after adrenalectomy in some animals and 3 months post-operatively in others. No diminution in glomerular filtration was noted except late in an adrenal crisis. An increase in the rate of reabsorption of water by the tubules was apparent during severe adrenal insufficiency.

SUBJECTS

There are two clinical conditions with which may be associated a profound depression of adrenal cortical activity and which are sufficiently

chronic in course to permit an accurate appraisal of changes in renal function. These are Addison's disease and pituitary hypofunction of the type designated as pan-hypopituitarism by Fraser, Albright and Smith (14). In this study ten patients with typical Addison's disease and six patients with chronic adrenal insufficiency secondary to pituitary hypofunction were investigated. Six persons acted as normal controls. None of the experimental subjects gave a history of having had acute or chronic nephritis. One patient, D. S., was suspected in 1935 of having renal tuberculosis. The diagnosis was not confirmed at that time or subsequently. A second patient, E. D., has had a persistent hypertension during the 2 years that he has been under observation. Otherwise no gross kidney disturbance was present in any subject.

The clinical methods for studying renal function were (a) determination of concentration of non-protein nitrogen of the serum, (b) testing ability to concentrate solids following abstinence from fluids for 12 hours, (c) urinary excretion of phenolsulphonphthalein dye 15 minutes after 1.0 cc. had been given intravenously, and (d) pyelography after intravenous administration of 20 cc. of diodrast. More precise measurements of kidney function were obtained from estimation of rate of clearance of inulin, creatinine, diodrast, glucose, sodium, and potassium.

The theoretical considerations which underlie the various clearance procedures will not be discussed. We have assumed (15, 16) that inulin clearance measures rate of glomerular filtration, that creatine clearance measures rate of glomerular filtration plus tubular excretion, that diodrast clearance at low iodine plasma levels measures maximum renal plasma flow, that diodrast clearance at high iodine plasma levels measures maximum tubular capacity for excreting diodrast, and that glucose clearance at high plasma levels measures maximum tubular capacity for reabsorbing glucose.

The patients with *Addison's disease* (Table I) who were studied presented the typical clinical picture. They were chosen without regard to duration of symptoms. None was known to be suffering from any serious complication of Addison's disease or from an associated malady at the time that the tests were performed. Except for W. H., the excretion of 17-ketosteroids was less than 10

TABLE I
Summary of clinical observations on ten patients with Addison's disease

Summary of clinical observations on ten patients with Addison's disease																						
Patient	Age	Sex	Duration of symptoms	Date	Blood pressure, lying				Serum				Urine				Intravenous pyelography	Calcification of adrenals by x-ray	Basal metabolic rate	Clinical state	Treatment	
					mm. Hg	mm. Hg	mm. Hg	mm. Hg	Sodium	Potassium	Nonprotein nitrogen	Whole blood sugar	Gravity	Albumin	Sediment	Excretion of phenolphthalein during first 15 minutes						mg. per 24 hours
H.M.	21	♀	1½	November 8, 1939	108/70	137.3	4.9	18	99	1.020	0	Negative	28	2.4	2.0	2.4	0	-27	Satisfactory.	Added sodium chloride. 3 weeks after beginning treatment with desoxycorticosterone acetate. 11 months after implantation of 1278 mgm. of desoxycorticosterone acetate. Added sodium chloride. 4 days after beginning treatment with desoxycorticosterone acetate. 15 months after implantation of 590 mgm. of desoxycorticosterone acetate. Added sodium chloride.		
W.H.	45	♂	6	February 15, 1941	92/68	118/70	140.6	4.0	31	106	1.018	0	Negative	34	14.4	1.3	30	0	-21	Satisfactory.	One week after beginning treatment with desoxycorticosterone acetate. Added sodium chloride.	
A.H.	50	♀	5	January 12, 1940	104/62	135.5	4.1	26	81	1.022	0	Negative	34	14.4	1.3	30	0	-21	Satisfactory.	One week after beginning treatment with desoxycorticosterone acetate. Added sodium chloride.		
A.O.	57	♂	6	January 31, 1940	120/80	138.4	4.2	21	106	1.022	0	Negative	34	14.4	1.3	30	0	-21	Satisfactory.	One week after beginning treatment with desoxycorticosterone acetate. Added sodium chloride.		
D.S.	47	♂	1	February 28, 1940	116/74	141.7	4.5	31	106	1.018	S.P. T.	0	18	3.2	4.7	3.6	0	-22	Satisfactory.	One week after beginning treatment with desoxycorticosterone acetate. Added sodium chloride.		
J.Coc.	47	♂	7	March 19, 1940	90/70	135.5	6.0	29	99	1.022	0	Negative	30	4.9	Poor function of right kidney	0	-20	Satisfactory.	One week after beginning treatment with desoxycorticosterone acetate. Added sodium chloride.			
				June 5, 1940	110/70	140.6	4.3	26	77	1.024	0	Negative	30	4.9	Poor function of right kidney	0	-21	Satisfactory.	5 weeks after beginning treatment with desoxycorticosterone acetate. 12 months after implantation of 491 mgm. of desoxycorticosterone acetate. 20 cc. adrenal cortical extract (Wilson's) intramuscularly each day for 5 days. 2 weeks after beginning treatment with desoxycorticosterone acetate. 1 year after implantation of 515 mgm. of desoxycorticosterone acetate. 20 cc. adrenal cortical extract (Wilson's) intramuscularly each day for 5 days. Added sodium chloride.			
				April 17, 1941	114/68	141.0	3.6	24	113	1.023	0	Negative	25	5.4	Normal	0	-1	Satisfactory.	1 year after implantation of 493 mgm. of desoxycorticosterone acetate. Added sodium chloride.			
				April 21, 1941	110/68	142.1	3.8	21	113	1.023	0	Negative	32	7.2	Normal	0	-10	Satisfactory.	Added sodium chloride.			
				May 3, 1940	120/70	137.0	4.0	32	64	1.023	0	Negative	30	4.5	Normal	0	-13	Satisfactory.				
				April 2, 1941	132/74	137.5	3.8	29	74	1.018	0	Negative	25	5.4	Normal	0	-10	Satisfactory.				
				April 8, 1941	120/70	138.2	4.1	27	80	1.016	0	Negative	30	4.5	Normal	0	-13	Satisfactory.				
J.Cob. 57	♂	1	June 23, 1940	110/65	126.6	5.0	48	87	1.024	0	Negative	25	2.0	Normal	0	-10	Symptoms of mild insufficiency.					
E.H. 34	♂	1½	July 19, 1940	110/65	128.0	4.7	28	87	1.024	0	Negative	35	7.8	Narrowed pelvis	0	-10	Symptoms of mild insufficiency.					
J.Col. 43	♂	4	July 17, 1941	120/78	141.0	4.0	20	83	1.024	0	Negative	28	7.2	Normal	0	-6	Satisfactory.					
				March 13, 1941	95/60	135.0	4.3	41	68	1.022	S.P. T.	0	28	7.2	Normal	0	-6	Satisfactory.				
J.L. 23	♂	1	July 23, 1941	110/60	140.8	4.2	16	84	1.020	0	Negative	28	7.2	Normal	0	-6	Satisfactory.					

mgm. per 24 hours. The normal range for excretion of this fraction, as determined by our laboratory, is from 15 to 25 mgm. per 24 hours for adult males, and from 10 to 20 mgm. per 24 hours for adult females. The low values presented in Table I are indicative of a severe depletion of adrenal cortical tissue. The basal metabolic rate was approximately 20 per cent below normal. Calcification of the adrenals was evident by x-ray in two patients. All of the patients except J. Coc. were studied first while they were on a high sodium chloride intake, but before receiving treatment with desoxycorticosterone acetate (Percorten). The first test on J. Coc. was performed 2 weeks after treatment with active material had begun. The tests were repeated on five patients from 2 to 6 weeks after institution of desoxycorticosterone acetate therapy. The requirements for desoxycorticosterone acetate were determined by the assay method described by Thorn and Firor (17). The active material was given first in oil over a period of 2 or more weeks. When the maintenance dose had been determined, pellets of desoxycorticosterone acetate were implanted. Five patients were re-investigated approximately a year later at the time that they were admitted for re-implantation of pellets. After this long interval of time they were beginning to note the effects of an inadequate assimilation of desoxycorticosterone acetate, but none was suffering from acute adrenal insufficiency. Two patients, whose electrolyte exchange had been restored by treatment with desoxycorticosterone acetate were studied before and after experimental administration of adrenal cortical extract (Wilson's). Twenty cc. of the extract were given daily intramuscularly in divided doses for 5 days.

Three Addison's patients have died since the data were collected. A. H. died from adenocarcinoma of the ovary with metastases. A post-mortem examination showed caseous adrenals as well as carcinoma. A second patient, J. Cob., was a bartender and renegade. He was most uncooperative, and according to friends, died at home after an alcoholic bout. No autopsy was performed. A third patient, H. M., died suddenly in April 1941. Two months previously she had been admitted to the hospital for a new supply of desoxycorticosterone acetate pellets. This admission was uneventful. One month before death she

was observed to be in good physical condition, her serum electrolytes were normal and the blood pressure was 128/82. She lived only 18 hours after the sudden onset of weakness and hyperpyrexia. On the last admission, the serum sodium concentration was 126 m.eq. per liter; nevertheless, subcutaneous edema was obvious. The concentration of blood sugar was 88 mgm. per 100 cc. A continuous slow infusion of dextrose solution was given during the 18 hours preceding death. No autopsy was performed. The other patients with Addison's disease are alive and in a satisfactory state of compensation.

The patients suffering from *pan-hypopituitarism* (Table II) had symptoms of myxedema and chronic adrenal insufficiency. By *pan-hypopituitarism* we mean a type of Simmond's disease in which the most conspicuous feature is hypothyroidism (18) rather than cachexia. All of the patients with this malady were moderately incapacitated in contrast to those with Addison's disease. Each patient was admitted to the hospital because of distressing symptoms and all had metabolic rates below minus 20 per cent. Previous to admission four patients had had at least one crisis, the symptoms of which had been attributed to myxedema, but in retrospect are believed to have been those of adrenal insufficiency. All were ambulatory without requiring specific treatment at the time the renal function tests were done.

A diagnosis of chronic adrenal insufficiency in these patients was based upon the following evidence: (a) the syndrome of *pan-hypopituitarism* is known to be associated with atrophy of the adrenals; (b) excretion of 17-ketosteroids in the urine was similar to patients with Addison's disease; (c) symptomatic improvement followed a high sodium chloride intake; (d) only small amounts of thyroid extract were tolerated. The blood pressure was normal in all except E. D. This patient was the only one who showed evidence of renal damage by routine clinical tests. Four of the patients are alive and their symptoms are moderately well controlled on a high salt diet supplemented by parenteral administration of male or female sex hormones. Patient C. O. died from diabetes mellitus and hemochromatosis, symptoms of which appeared after renal function studies had been performed. Patient E. B. died following surgical removal of a suprasellar cyst. Atrophy

TABLE II
Summary of clinical observations on six patients with pan-hypopituitarism

Patient	Age	Sex	Duration of symptoms years	Date	Blood pressure, lying mm. Hg	Serum			Whole blood sugar mgm. per 100 cc.	Urine					Intravenous pyelography	Basal metabolic rate per cent
						Sodium m. eq. per liter	Potassium m. eq. per liter	Nonprotein nitrogen mgm. per 100 cc.		Maximum specific gravity	Albumin	Sediment	Excretion of phenolphthalein during first 15 minutes per cent	17-Ketosteroids mgm. per 24 hours		
N.W.....	54	♂	10	November 13, 1939	110/60	138.7	4.1	20	96	1.020	0	Negative		0.0		-40
				December 15, 1939	100/65			21	80							-40
C.O.....	53	♂	2	December 20, 1939	122/88				286	1.022	0	Rare white blood cell		2.0		-39
D.A.....	29	♀	5	January 30, 1940	100/70	138.5		30	111	1.022	0	Negative		2.4		-23
E.D.....	70	♂	13	May 11, 1940	170/85	132.3	4.3	29	74	1.025	0	Negative	13	3.0	Normal	-19
				November 25, 1940	190/120				80	1.012	S.P.T.	Rare cast	12	3.3	Normal	-34
E.B.....	27	♂	1	July 19, 1941	110/70	138.4	4.0	20	82	1.017	0	Negative	34	1.8	Normal	-26
B.L.....	18	♂	1	August 4, 1941	104/72	140.5	4.4	18	56	1.022	0	Negative	28	3.2	Normal	-35

of the adrenals was apparent at postmortem examination in both patients.

Although the term adrenal insufficiency is used frequently throughout this communication, none of the observations was made during an adrenal crisis. The study was planned in order to gather renal function data on ambulatory patients whose clinical symptoms were satisfactorily controlled. When the words adrenal insufficiency are used, therefore, it implies nothing more than an anatomical deficiency of adrenal cortical tissue. By this definition all patients with typical Addison's disease or pan-hypopituitarism, irrespective of apparent adequacy of treatment, are suffering from adrenal insufficiency. This statement is not intended to imply that the current drugs used in the treatment of Addison's disease are unsatisfactory. On the contrary, they have altered the clinical course of Addison's disease tremendously. Nevertheless, we believe that in most patients even this treatment does not provide for complete restoration.

During the period that these studies were in progress, seven additional patients with Addison's disease were admitted to the hospital in an acute adrenal crisis. Not one of these patients was thought to be a suitable subject for clearance tests since we were interested in renal function in chronic adrenal insufficiency. Microscopic examination of the kidneys from three of these patients is discussed under Pathological Observations.

METHODS

Most of the data were collected while the patients were in the research ward of the Massachusetts General Hospital. Approximately 25 grams of inulin were given intravenously for the determination of inulin clearance. For the determination of renal blood flow, the concentration of diodrast iodine in the serum was maintained at approximately 1 mgm. per 100 cc. Maximum tubular excretory capacity was determined with diodrast iodine levels in the serum, decreasing each 10-minute period from approximately 50 mgm. per 100 cc. to 25 mgm. per 100 cc. Maximum tubular reabsorption capacity was determined with rising serum glucose levels above 400 mgm. per 100 cc. The inulin, creatinine, sodium and potassium clearance and diodrast clearance at low plasma levels represent an average of 3 or more 10-minute collection periods. The data for diodrast and glucose clearance at high plasma levels are the average for 3 periods or less. All of the clearance tests are corrected to a body surface area of 1.73 sq.m. (16).

The tests were begun while the subjects were in a basal state except as regards consumption of water. One liter of fluid was allowed 12 hours and 2 hours, respectively, before each test. All urine specimens were collected by a urethral catheter. A sample of blood was taken at the halfway time in most periods. When this was not done, the concentration of constituents of serum was interpolated from data collected during periods immediately before and after. Venous blood was taken for all determinations except glucose. When concentration of this constituent was desired, arterial blood was taken from the brachial artery. Timing was done by means of a stop watch. Iodine was determined according to the method described by Smith and associates (16). The methods used for determination of the other constituents in blood and urine have been described (19).

RESULTS

Addison's disease

In the summary given in Table I of the clinical observations on patients with Addison's disease, it is apparent that at the time most of the tests were performed, clinical evidence of acute adrenal insufficiency was lacking. At the time of the third test on H. M., at the time of the first test on E. H., and at the single test on J. Cob., symptoms of mild adrenal insufficiency were evident. The serum sodium concentrations during these tests tended to be less than 135 m.eq. per liter. In all determinations except the first on D. S., the serum potassium was within the range for normals. Laboratory and clinical evidence of hypoglycemia was absent in each patient. Previous to our studies, treatment in all patients except J. Coc. consisted only of a high salt intake and other conservative measures.

Examination of kidney function by routine clinical procedures showed no striking or constant variation from the normal. A total of 91 separate determinations, which include maximum specific gravity, phenolsulphonphthalein excretion, concentration of nonprotein nitrogen in the serum, albumin in the urine, urinary sediment and intravenous pyelography, are reported in Table I. Of these only 16, or less than 18 per cent, were outside the average range for normals. Albumin was noted at 2 examinations, blood cells or casts at 4 examinations. Eight of the ten patients were able to concentrate urine to 1.020 during at least one examination. The nonprotein nitrogen was above 35 mgm. per 100 cc. in only two instances. The intravenous pyelogram was normal in all except two patients. All except one subject was able to excrete more than 25 per cent phenolsulphonphthalein dye during the first 15 minutes after injection.

In contrast to these data, the rate of formation of glomerular filtrate as determined by *inulin clearance* was below normal at the first observation in each patient (Table III). Thus, W. H. on December 9, 1939, had an inulin clearance of only 82 cc. per minute; yet he was able to concentrate urine to 1.024 and excreted 30 per cent of phenolsulphonphthalein dye during the first 15 minutes after intravenous injection. The values in nine

patients ranged between 56 cc. and 97 cc. The average was 77 cc. In our investigations of inulin clearance we have chosen arbitrarily 100 cc. of plasma per minute as the lower limit for a normal person and 120 cc. as an average normal value. These data are similar to those reported by Goldring and associates (20).

Following the first series of renal function studies, desoxycorticosterone acetate in oil was administered and, later, pellets of this substance were implanted. Subsequently, an increase in rate of formation of glomerular filtrate was apparent in the five patients on whom this function was studied. The increases averaged 32 per cent, a significant gain. Simultaneous clinical improvement was more subtle than obvious since the patients were in a relatively satisfactory clinical condition at the time of the initial tests. Following implantation of pellets for one year or longer, the improvement in renal function noted shortly after institution of desoxycorticosterone acetate therapy had regressed in all except E. H. Three of the five patients on whom these tests were repeated showed an inulin clearance below that observed at the time of the first admission to the hospital. This result is not surprising since the life of the pellets used in this study is approximately 12 months and the amount of drug available from this source decreases rapidly after one year. It should be stressed, however, that except for H. M. no clinical symptoms of acute adrenal insufficiency were apparent and the patients were readmitted because we had advised it. Each patient believed that clinical improvement which developed month by month following desoxycorticosterone acetate therapy had been maintained in a large part.

The effect of administration of adrenal cortical extract (Wilson's) upon kidney function was investigated in two patients. Each was given 20 cc. of the material daily in divided doses intramuscularly for 5 days. The extract was given to ascertain whether it would have any demonstrable effect upon inulin clearance. No striking improvement in clinical state was anticipated because this was satisfactory before administration of this material. The experiment was negative in both subjects. It was concluded that adrenal cortical extract in the doses given is unable to restore impaired renal function in patients with Addison's

disease beyond that achieved by desoxycorticosterone acetate.

Creatinine clearance was determined simultaneously with inulin clearance in most instances. A normal person excretes approximately 25 per cent of the total creatinine by means of tubular excretory activity and the remainder by glomerular filtration. The patients with Addison's disease in our series excreted slightly more than 30 per cent of the total by the tubules and proportionately less by glomerular activity. This is indirect evidence that glomerular filtration in Addison's disease is depressed to a greater extent than is renal blood flow; the latter supplying creatinine-containing blood for tubular excretion.

Further evidence to support the assumption that in Addison's disease glomerular filtration suffers more than does renal blood flow was deduced from measurements of *diodrast clearance at low plasma*

levels (Table III). Effective renal blood flow may be calculated from diodrast clearance at low plasma levels and cell volume. This function was not determined in many patients, but the collected observations show proportionately less depression below normal than do inulin clearances. Renal plasma flow averaged 465 cc. per minute in 10 tests on the Addisonian patients. The average for the normal subjects was 690 cc. per minute. Administration of adrenal cortical extract did not affect this function.

The ratio of glomerular filtration to renal plasma flow has been designated *filtration fraction* by Smith. In five normal subjects this averaged 21.5 per cent. In the Addison's patients it averaged 15.7 per cent. A diminution in filtration fraction may be accomplished by a decrease in blood pressure or a diminution of tone of efferent arterioles of the nephron. Since the blood pres-

TABLE III
Renal function observations on ten patients with Addison's disease

Patient	Date	Body surface	Average urine flow during test	Cc. of plasma cleared per minute. Average of 3 or more periods.					Effective renal blood flow	Dio-drast-iodine Tm.	Glu-cose Tm.	Filtration fraction
				Inulin	Creati-nine	Sodi-um	Potas-ium	Dio-drast-iodine				
		sq. m.	cc. per minute						cc. per minute	mgm. per minute	mgm. per minute	per cent
H.M.	November 8, 1939	1.40	6.6	97	152	6.8	27.2					
	November 28, 1939	1.41	7.3	102	145	6.5	35.3					
	February 15, 1941	1.34	0.6	55		0.9		442	692	36		12.5
W.H.	December 9, 1939	1.63	5.7	82	118	0.9	8.9					
	December 23, 1939	1.64	11.5	100	159	2.9	10.1					
	March 3, 1941	1.68	3.9	57		0.7	7.8	592	924	42		9.7
A.H.	January 12, 1940	1.42	1.9	70	122	1.7	9.4					
	January 31, 1940	1.44	2.5	106	125	4.0	12.8					
A.O.	February 13, 1940	1.48	1.3	82	115	0.6	7.7					
	February 28, 1940	1.52	1.1	91	139	1.4	9.2					
D.S.	March 19, 1940	1.42	4.0	56	69	2.8	6.7					
	June 5, 1940	1.46	2.0	95	132	0.5	14.4					
	April 17, 1941	1.49	2.8	78				358	560		186	21.8
	April 21, 1941	1.49	1.8	72				376	586		188	19.2
J.Coc.	May 3, 1940	1.65	1.5	92	132	1.1	7.8					
	April 4, 1941	1.67	2.7	70		0.3		416	614			16.8
	April 9, 1941	1.68	3.2	68		0.9		366	538	23		18.9
J.Cob.	June 23, 1940	1.61	2.4	82	103	1.8	10.8					
E.H.	July 19, 1940	1.85	3.1	68	116	1.2	12.1	388	682	29	153	17.5
	July 17, 1941	1.84	1.0	80		1.0	6.0	504	840	36		15.9
J.Col.	March 13, 1941	1.86	1.0	81		0.5		690	1110			11.8
J.L.	July 28, 1941	1.64	1.8	74			2.6	520	850	35		14.2

tures were essentially normal, it is assumed that the decreased fraction was produced by efferent arteriolar relaxation.

The observations on maximum tubular capacity for excretion of diodrast (*diodrast Tm.*) and maximum tubular capacity for reabsorption of glucose (*glucose Tm.*) are too few for one to draw conclusions. It may be stated, however, that tubular excretory capacity appears to be maintained remarkably well, while capacity for reabsorbing glucose is impaired seriously. Reabsorption of water by the tubules was calculated for five patients before and after treatment with desoxycorticosterone acetate. The percentile reabsorption was unchanged in three patients, increased in one and decreased in one. No significant change was noted in the two patients who received adrenal cortical extract. Our data, therefore, do not confirm the conclusions of Gersh and Grollman (13) and Silvette and Britton (21) that tubular reabsorption of water is increased in animals in adrenal insufficiency. Increased tubular reabsorption of water would be a rather unexpected finding since glomerular filtration is depressed. Furthermore, in acute adrenal insufficiency increased urinary output accompanies development of symptoms.

The exchange of sodium, chloride and potassium by the kidney was calculated as cc. of plasma cleared per minute and recalculated to determine per cent reabsorption by the tubules according to

the formula $\left(1 - \frac{\text{sodium clearance}}{\text{inulin clearance}}\right) \times 100$. In

employing the term clearance for electrolyte exchange, we have assumed that it implies and embodies a process similar to the clearance of substances such as urea, phosphate, and urate. Inherent aspects of electrolyte clearance include appearance of these substances in the glomerular filtrate in the same concentration as they exist in plasma, and reabsorption in part by tubular cells as the glomerular filtrate passes through the tubular lumina. We have assumed that electrolytes are not excreted by the tubules.

The electrolyte clearances were determined because the statement has been made that the tubule cannot absorb adequate quantities of sodium in adrenal insufficiency (22, 23). Additional data on

this subject seemed desirable. Normal persons on a sodium chloride intake of from 6 to 10 grams per day will have a "sodium clearance" of from 1 to 5 cc. of plasma per minute. All of the Addison's patients except H. M. had a sodium clearance within this range or below. During the period of the test, therefore, no dissipation of sodium was apparent. Similar conclusions were reached by Schäfer (24) who employed a different technique for the study of renal activity of the adrenalectomized dog and cat. Our conclusions are not intended to imply that a dissipation of sodium does not occur in adrenal insufficiency (25). In fact these clearance data may be interpreted as confirming the assumption that, during the tests, none of the patients was suffering from acute adrenal insufficiency. An alternate explanation for our inability to detect any dissipation of sodium is that these studies on renal function are not sufficiently precise to detect subtle variations in sodium exchange. The results obtained from the calculation of tubular reabsorption of chloride are scattered and are not conclusive.

The data on potassium clearance appear to be more definitive. Each of the first five patients studied showed an increased clearance of potassium following treatment with desoxycorticosterone acetate. This was an expected result since this substance promotes elimination of potassium as well as retention of sodium. The increased excretion of potassium is produced mainly by an increase in glomerular filtration. Calculation of the amount reabsorbed by substituting potassium clearance in the above-mentioned formula gives scattered results just as for sodium.

Adrenal insufficiency secondary to pan-hypopituitarism

All of the patients with pituitary hypofunction showed a profound depression in inulin clearance (Table IV). The average for the group was approximately one-half that for normal controls. Creatinine clearance showed a smaller percentile decrease from normal, as was observed in patients with Addison's disease. More than 30 per cent of the creatinine excreted was by means of tubular activity. Diodrast clearance at low plasma levels was determined on two patients. The filtration fractions were 21.1 and 19.1 per cent, respectively.

TABLE IV

Renal function observations on six patients with pan-hypopituitarism

Patient	Date	Body surface sq. m.	Average urine flow cc. per minute during test	Cc. of plasma cleared per minute. Average of 3 or more periods.					Diodrast-iodine Tm. mgm. per minute	Filtration fraction per cent
				Inulin	Creatinine	Sodium	Potassium	Diodrast-iodine		
N.W.	November 13, 1939	1.35	0.9	49	70	0.3	16.9			
	December 15, 1939	1.37	1.8	40		0.6	9.0			
C.O.	December 20, 1939	1.71	9.6	70	118	1.0	8.5			
D.A.	January 30, 1940	1.57	8.0	61	89	0.6	21.3			
E.D.	May 11, 1940	1.70	11.4	63	91	7.4	11.8	204	16.6	21.1
	November 25, 1940	1.81	7.6	43		5.2	13.3			
E.B.	July 19, 1941	1.64	5.1	61				320	27.5	19.1
B.L.	August 4, 1941	1.64	9.8	77					43.9	

Normal controls

The effect of desoxycorticosterone acetate upon renal activity was studied in two normal subjects, B. C. and B. D. Each subject was given daily 10 mgm. of the substance in oil for 7 days. Clearance studies before and after administration to B. C. were similar. Before-administration studies were not done on B. D. but after-treatment studies were within the average range for normals.

PATHOLOGICAL OBSERVATIONS

One of our interests in pursuing this investigation of renal function in adrenal insufficiency was the reported absence of structural damage in the kidney of patients dying from Addison's disease (2, 26, 27, 28, 29). One might assume, therefore, that the changes in the kidney were "functional" and reversible if the adrenal insufficiency could be controlled. A comprehensive study of

TABLE V

Renal function observations on six control subjects

Patient	Age	Sex	Date	Body surface sq. m.	Average urine flow during test cc. per minute	Cc. of plasma cleared per minute. Average of 3 or more periods					Diodrast-iodine Tm. mgm. per minute	Filtration fraction per cent	Remarks
						Inulin	Creatinine	Sodium	Potassium	Diodrast-iodine			
R.J.	40	♂	January 4, 1939	1.63	112	153	2.0	25.4					Normal regimen. 7 grams of potassium chloride during 3 hours before test. Low potassium diet for 8 days. Low potassium diet for 11 days. 30 grams sodium chloride during 48 hours before test.
			January 3, 1940	1.68	16.6	103	131	5.4	75.1				
			January 6, 1940	1.68	17.2	104	145	3.1	78.4				
			January 16, 1940	1.67	16.8	95	142	3.2	11.4				
			January 19, 1940	1.68	7.5	98	142	5.1	12.3				
B.C.	19	♂	November 14, 1940	1.74	8.5	183	254	4.9	16.1	730	66.4	25.0	Normal regimen. 10 mgm. desoxycorticosterone acetate intramuscularly each day for 7 days.
			December 10, 1940	1.74	2.0	190	269	1.7	20.2	710	65.4	26.7	
D.D.	54	♂	November 22, 1940	1.99	3.4	116	164	3.4	8.8	530	43.8	21.9	10 mgm. desoxycorticosterone acetate intramuscularly each for day 7 days.
L.C.	32	♀	December 4, 1940	1.64	8.8	122	179	2.8	27.4	676	54.5	18.1	Normal regimen.
R.S.	18	♂	July 27, 1940	1.77	3.5	185	210	3.1		570	44.0	21.3	Normal regimen.
F.M.	23	♀	August 7, 1940	1.61	9.2	139	190	1.1		675	48.0	20.6	Normal regimen.

postmortem material was reported by Guttman (30) in 1929. In a statistical study of 566 autopsied cases of Addison's disease collected from the literature, less than 10 per cent showed morphologic changes in the kidneys sufficient to justify an anatomic diagnosis of renal disease. Approximately one-half of this number showed tuberculosis of the kidney; the remainder showed among other conditions acute and chronic nephritis and pyelonephritis.

Somewhat different findings were reported by Barker (31) in thirty-one autopsied cases. Ten of this number showed tubular atrophy which consisted of "flattening of the epithelial cells and diminution in the amount of cytoplasm. The tubular lumina usually appeared diminished in diameter with intertubular edema. Occasionally, fat was evident in the tubular cells." Barker interpreted these changes as the analogue of a toxic nephrosis which had been produced by hypotension and anoxemia. Similar pathological findings have been observed in adrenalectomized animals. Fat deposits in the epithelium of the collecting tubules have been noted in the cat (5, 32) and general swelling of the tubular cells in the dog (33). It is of interest that Simpson and Korenchevsky (34) prevented the development of degenerative changes in the tubules of rats following adrenalectomy by the use of adrenal cortical extract. No mention was made of time allowed for the development of degenerative changes or the amount of extract necessary for prevention of changes.

In our series of patients none of them gave a history of having had any renal disturbance and a diagnosis of acute or chronic nephritis, nephrosis, or pyelonephritis was not presumed in any. D. S. had changes by intravenous pyelogram which suggested unilateral tuberculosis of the kidney. He has been followed over a period of more than 4 years and the diagnosis has never been confirmed either by medical or urological consultants. Injection of a sample of urine into a guinea pig did not lead to tuberculosis in the animal.

Finally, we are able to report on postmortem material from three patients studied in this series as well as three other patients who died from Addison's disease. The microscopic examinations were made by Dr. B. M. Castleman. The kidneys of A. H. at autopsy showed minimal structural

changes consistent with pyelitis and pyelonephritis. It is significant that our studies which were done 5 months before death from carcinoma showed a low normal inulin clearance following implantation of pellets. If the pyelonephritis were playing a large rôle in depression of glomerular filtration, it is assumed that desoxycorticosterone acetate could not have restored this function to normal. It is concluded that glomerular filtration on entry was not depressed because of pyelonephritis, but was an integral part of the adrenal disturbance. C. O. had normal kidneys except for small deposits of hemosiderin. It is not believed that the hemosiderin deposits had interfered seriously with renal function during life. The kidneys of E. B., as well as those of three other patients who died from Addison's disease during the past 2 years, but who were not suitable patients for clearance studies, appeared normal.

DISCUSSION

The capacity of the kidney has been investigated in patients with Addison's disease and in patients with adrenal cortical atrophy secondary to pituitary hypofunction. Both groups of patients were studied at a time when clinical symptoms of acute adrenal insufficiency were absent. The data reported, therefore, are to be interpreted as an integral part of controlled adrenal insufficiency rather than severe adrenal depletion. Renal function was studied by the clinical tests usually applied and by more quantitative measurements of specific renal activity. Routine clinical tests in the majority of instances were normal. On the other hand, precise measurement of renal function by the clearance tests recently described by Smith and associates showed considerable impairment.

Rate of formation of glomerular filtrate as measured by inulin clearance was depressed significantly in all patients before treatment. From 1 to 4 weeks following treatment with desoxycorticosterone acetate, the patients with Addison's disease showed partial restoration of glomerular filtration. One year or more following implantation of pellets of desoxycorticosterone acetate and, in spite of continued clinical improvement, regression of glomerular filtration rate had taken place. Renal plasma flow as measured by diodrast

clearance at low plasma levels showed a smaller percentile depression than did inulin clearance. The ratio of inulin clearance to diodrast clearance, *i.e.*, filtration fraction, was considerably below normal. Of all the measured functions, maximum tubular capacity for excreting diodrast at high iodine plasma levels was affected least. Maximum tubular capacity for reabsorbing glucose at high plasma levels was most affected.

There are several factors which might be responsible for the pathogenesis of renal impairment in symptomatically controlled adrenal insufficiency. These are (1) vasomotor unresponsiveness and hypotension, (2) increase in concentration of serum protein, (3) decrease in metabolic rate, (4) structural changes in the kidney, (5) non-specific result of a chronic disease, and (6) lack of some specific action of one or more adrenal cortical hormones.

Diminution of blood pressure may be suspected because hypotension might produce many of the observed changes. In acute adrenal insufficiency, a significant depression in renal function undoubtedly may be attributed to hypotension. In our studies, however, blood pressures were normal in most instances. Furthermore, if hypotension had been responsible, renal plasma flow should have suffered quite as much as inulin clearance, instead of the reverse. The data suggest a diminution in efferent arteriolar tone with less impairment of tubular activity than rate of formation of glomerular filtrate. This is consistent with the clinical findings of a poorly functioning vasomotor system.

Dehydration with increase in colloid osmotic pressure was not seriously implicated because the concentrations of serum protein were within the range for normal.

A decrease in metabolic rate has not been entirely excluded as being responsible for the observed effects. In Addison's disease, as well as in adrenal insufficiency secondary to pituitary hypofunction, the metabolic rate is depressed. If the averages of the two groups are compared, the patients with pituitary hypofunction had a greater depression of kidney function as well as a greater depression in metabolic rate. Since the hormone produced by the thyroid has an effect upon cellular activity generally, it is reasonable to

assume that depressed tubular activity may follow inadequate elaboration of it.

Structural changes in the kidney are observed infrequently in Addison's disease and cannot be held responsible for the functional impairment.

We cannot refute with certainty the supposition that the observed changes are the effect of a chronic disease *per se*. Signs of grave renal insufficiency which accompany acute adrenal insufficiency argue for a more specific relationship.

Maintenance of the integrity of the kidney by hormones of the adrenal cortex was postulated by Marshall and Davis in 1914 (5). Such a postulation is not unreasonable and partial restoration in inulin clearance immediately following treatment with desoxycorticosterone acetate supports it. The failure of kidney function to be maintained a year after implantation of pellets and the absence of improvement in renal function in Addison's disease following administration of adrenal cortical extract negate the postulation.

In conclusion, the correct explanation of the pathogenesis of renal impairment may be multiple rather than single. A combination of all or of most of the hypotheses would be in keeping with the statement made in the introduction of the paper, *i.e.*, adrenal insufficiency is a complex phenomenon.

SUMMARY

Studies of kidney function have been pursued in ten patients with chronic adrenal insufficiency associated with Addison's disease and in six patients with chronic adrenal insufficiency associated with pan-hypopituitarism. All of the patients except two gave a negative history for acute or chronic renal disease. In most instances the patients were well compensated and symptoms of severe adrenal insufficiency were not present immediately before, during or after any of the tests. The function tests included accepted clinical procedures, as well as clearance of inulin, creatinine, diodrast, glucose, sodium, chloride and potassium.

The clinical tests for renal disease were normal in most patients. On the other hand, more precise tests showed evidence of impairment of all measured aspects of renal function in all patients at most examinations. Rate of formation of glomerular filtrate and tubular reabsorptive ca-

capacity for glucose were affected most. Renal plasma flow was affected less and tubular capacity for excreting diodrast was affected least. The filtration fraction was depressed below normal. Administration of desoxycorticosterone acetate corrected partially, but temporarily, these deficiencies. Administration of adrenal cortical extract had no demonstrable action upon the measured functions. Administration of desoxycorticosterone acetate to two normal persons was without demonstrable effect upon renal activity.

The pathogenesis of these aberrations is assumed to be "functional" in so far as no structural changes are consistently observed in the kidneys of patients who have died from adrenal insufficiency. Vasomotor unresponsiveness, a decrease in metabolic rate, a nonspecific effect of a chronic disease, and a lack of specific action by the adrenal cortical hormones may each contribute.

BIBLIOGRAPHY

- Loeb, R. F., Atchley, D. W., and Parson, W., The significance of certain chemical abnormalities found in the blood in Addison's disease. *Tr. A. Am. Physicians*, 1937, 52, 228.
- Sicard, J. A., and Haguénau, Dosage de l'Uréé Sanguine des Addisoniens. *Bull. et mem. Soc. med. d. Hôp. de Paris*, 1914, 37, 902.
- Gaillard, L., Insuffisance Surrénale et Azotémie. *Bull. et mem. Soc. med. d. Hôp. de Paris*, 1914, 37, 272.
- Porak, R., and Chabanier, H., Altération de la Sécrétion Renale Après l'Albation des Glandes Surrénales. *Compt. Rend. Soc. de Biol.*, 1914, 77, 440.
- Marshall, E. K., Jr., and Davis, D. M., The influence of the adrenals on the kidneys. *J. Pharmacol. and Exper. Therap.*, 1916, 8, 525.
- Stahl, J., Atchley, D. W., and Loeb, R. F., Observations on adrenal insufficiency. *J. Clin. Invest.*, 1936, 15, 41.
- Rowntree, L. G., Studies in Addison's disease. *J. A. M. A.*, 1925, 84, 327.
- Rowntree, L. G., and Snell, A. M., A Clinical Study of Addison's Disease. W. B. Saunders Co., Philadelphia, 1931.
- Rosenow, G., Ueber die Nierenfunktion bei der Addison'schen Krankheit. *Med. Klin.*, 1925, 21, 202.
- Rehberg, P. B., Studies on kidney function. II. The excretion of urea and chloride analyzed according to a modified filtration-reabsorption theory. *Biochem. J.*, 1926, 20, 461.
- Margitay-Becht, E., and Gömöri, P., Die Nierenfunktion bei der Addison'schen Krankheit. *Ztschr. f. d. ges. exper. Med.*, 1938, 104, 22.
- McCance, R. A., Medical problems in mineral metabolism. II. Sodium deficiencies in clinical medicine. *Lancet*, 1936, 1, 765.
- Gersh, I., and Grollman, A., Kidney function in adrenal cortical insufficiency. *Am. J. Physiol.*, 1939, 125, 66.
- Fraser, R., Albright, F., and Smith, P. H., Carbohydrate metabolism. The value of the glucose tolerance test, the insulin tolerance test, and the glucose-insulin tolerance test in the diagnosis of endocrinologic disorders of glucose metabolism. *J. Clin. Endocrinology*, 1941, 1, 297.
- Shannon, J. A., and Smith, H. W., The excretion of inulin, xylose and urea by normal and phlorizinized man. *J. Clin. Invest.*, 1935, 14, 393.
- Smith, H. W., Goldring, W., and Chasis, H., The measurement of the tubular excretory mass, effective blood flow and filtration rate in the normal human kidney. *J. Clin. Invest.*, 1938, 17, 263.
- Thorn, G. W., and Firor, W. M., Desoxycorticosterone acetate therapy in Addison's disease. Clinical considerations. *J. A. M. A.*, 1940, 114, 2517.
- Means, J. H., Hertz, S., and Lerman, J., The pituitary type of myxedema or Simmonds' disease masquerading as myxedema. *Tr. A. Am. Physicians*, 1940, 55, 32.
- Coombs, F. S., Pecora, L. J., Thorogood, E., Consolazio, W. V., and Talbott, J. H., Renal function in patients with gout. *J. Clin. Invest.*, 1940, 19, 525.
- Goldring, W., Chasis, H., Ranges, H. A., and Smith, H. W., Relations of effective renal blood flow and glomerular filtration to tubular excretory mass in normal man. *J. Clin. Invest.*, 1940, 19, 738.
- Silvette, H., and Britton, S. W., Renal function in normal and adrenalectomized opossums and effects of post-pituitary and cortico-adrenal extracts. *Am. J. Physiol.*, 1938, 121, 528.
- Darrow, D. C., Renal function in adrenal insufficiency. *Connecticut State M. J.*, 1940, 4, 393.
- Darrow, D. C., and Harrison, H. E., The chemical composition of tissues in adrenal insufficiency. *J. Biol. Chem.*, 1938, 123, xxvii.
- Schäfer, W., Die Nierenfunktion nach Nebennierenextirpation. *Ztschr. f. d. ges. exper. Med.*, 1933, 90, 552.
- Harrison, H. E., and Darrow, D. C., Renal function in experimental adrenal insufficiency. *Am. J. Physiol.*, 1939, 125, 631.
- Stahl, J., Kuhlmann, D., and Urban, M., Les Troubles de la Sécrétion Uréique au cours de l'Insuffisance Surrénalienne. *Compt. rend. Soc. de biol.*, 1928, 127, 1283.
- Mainzer, F., Ueber die Störung der "Nierenfunktion" bei Addison'schen Krankheit. *Schweiz. med. Wchnschr.*, 1937, 67, 31.
- Joelson, J. J., and Shorr, E., The relation of the suprarenals to cholesterol metabolism. *Arch. Int. Med.*, 1924, 34, 841.

29. Mozer, J. J., De l'influence de la Surrénale sur le Fonctionnement du Rein. *Presse med.*, 1929, 37, 156.
30. Guttman, P. H., Addison's disease. A statistical analysis of five hundred and sixty-six cases and a study of the pathology. *Arch. Path.*, 1930, 10, 742.
31. Barker, N. W., The pathologic anatomy in twenty-eight cases of Addison's disease. *Arch. Path.*, 1929, 8, 432.
32. Hartmann, F. A., MacArthur, C. G., Gunn, F. D., Hartman, W. E., and MacDonald, J. J., Kidney function in adrenal insufficiency. *Am. J. Physiol.*, 1927, 81, 244.
33. Banting, F. G., and Gairns, S., Suprarenal insufficiency. *Am. J. Physiol.*, 1926, 77, 100.
34. Simpson, S. L., and Korenchevsky, V., Histological changes in the kidneys of adrenalectomized rats. *J. Path. and Bact.*, 1935, 40, 483.

STUDIES ON THE INFLUENCE OF VITAMIN A AND VITAMIN C ON CERTAIN IMMUNOLOGICAL REACTIONS IN MAN^{1,2}

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There has been considerable difference of opinion concerning the influence of the vitamins upon resistance to infection (1). Many of the published reports, based to a great extent upon studies in experimental animals, have tended to suggest that vitamin A and vitamin C are especially important in maintaining resistance (2). The rôle of these vitamins in the resistance of human subjects to infection has not been satisfactorily established.

The present study was undertaken to reinvestigate the influence of vitamin A and vitamin C upon a series of immunological reactions in patients receiving diets adequate in all other factors except the specific vitamin, the influence of which was to be studied. The following immunological phenomena were studied: (1) the capacity of the patient's nasal secretions to inactivate influenza virus (3); (2) the titer in the patient's blood serum of neutralizing antibodies for influenza virus; (3) the activity of lysozyme in the nasal secretions; (4) the titer of complement in blood serum; and (5) the phagocytic activity for pneumococci of polymorphonuclear neutrophilic leukocytes in whole, heparinized blood.

METHODS

Selection and care of patients

The five subjects were patients in the wards of the Third (New York University) Medical Division of Bellevue Hospital. Three of them had been admitted to the hospital for some minor complaint; one patient had cirrhosis of the liver and the other had ankylosing spondylitis. All patients were under the observation of a special nurse.

Dietary measures

Each patient received a diet which was deficient either in vitamin A or vitamin C but which was adequate in

¹ This research was aided by a grant from the Josiah Macy, Jr. Foundation.

² The cod liver oil concentrate used in this study was supplied by the Lederle Laboratories, Inc.

calories and all other foodstuffs. In addition, each patient received a daily supplement of 10 to 20 mgm. thiamine chloride, 50 to 100 mgm. nicotinic acid, 1 to 2 mgm. riboflavin and 15 to 30 grams of dried brewers' yeast. Furthermore, patients who were on the diet deficient in vitamin A received 100 to 200 mgm. of ascorbic acid daily; patients on the diet deficient in vitamin C received 100,000 to 300,000 U.S.P. units of vitamin A in the form of a cod liver oil concentrate daily.

The diets were prepared by the University dietitian. The diet deficient in vitamin A contained not more than 300 U.S.P. units of vitamin A per day (Table I). The

TABLE I

Vitamin A deficient diet

Breakfast
Fruit
Cereal
Skimmed milk, one pint
Bread, three slices
Coffee
Lunch
Lean beef or fish
Potato, rice or spaghetti
Skimmed milk, one pint
Bread, three slices
Plain pudding, Jello, fruit or ice cream
Supper
Lean beef or cottage cheese
Rice, potato or spaghetti
Skimmed milk, one pint
Bread, three slices
Dessert, same as for lunch

Butter, eggs and vegetables were excluded from the diet and Mazola oil was used to cook the meat and fish.

The only significant source of vitamin A in the diet was the milk which was skimmed and contained not more than 20 U.S.P. units of vitamin A per 100 cc.

diet deficient in vitamin C contained about 5 mgm. of vitamin C per day (Table II).

Vitamin determinations

Weekly or bi-weekly determinations of the amount of vitamin A, carotene and vitamin C in the blood plasma of each patient were made. The amount of vitamin C in the white blood cell-platelet layer was determined at intervals. All of the determinations, except where specifically indicated otherwise, were made on blood ob-

TABLE II

Vitamin C deficient diet

Breakfast

Cereal, with sugar
Skimmed milk, one pint
Egg, one only
Bread, three slices, with butter

Lunch

Lean beef or fish
Rice, spaghetti or noodles
Skimmed milk, one pint
Bread, three slices, with butter
Plain pudding, custard, Jello or ice cream

Supper

Cheese, egg or lean beef
Rice, noodles or spaghetti
Skimmed milk, one pint
Bread, three slices, with butter
Dessert, same as for lunch

Fruit and vegetables were excluded from the diet.

tained from the patient 24 hours after the last administration of vitamin A or vitamin C.

The concentration of vitamin A in the plasma was determined in the photoelectric colorimeter by the method reported by Kimble (4). The amount of carotene in the plasma was determined in the photoelectric colorimeter by the method described by Stueck *et al.* (5).

The content of vitamin C in the plasma was determined in the photoelectric colorimeter by the method of Mindlin and Butler (6), using methylene blue as the indicator in place of 2, 6 dichlorophenol indophenol. The concentration of vitamin C in the white blood cell-platelet layer was measured by the method of Butler and Cushman (7).

It has been reported by others (4) and substantiated by Ralli *et al.* (8) that in normal adults the level of vitamin A in the plasma, as determined in the photoelectric colorimeter, varies from 88 to 220 U.S.P. units per 100 cc. and the amount of carotene in the plasma varies from 0.080 to 0.280 mgm. per cent.

In normal adults the amount of vitamin C in the plasma is above 0.4 mgm. per cent. The concentration of the vitamin in the white blood cell-platelet layer is about 25 mgm. per 100 grams (9). The best test at present available for detecting a profound depletion of the stores of vitamin C in the tissues of humans, prior to the appearance of the symptoms of scurvy, is the determination of the amount of vitamin C in the white blood cell-platelet layer. A decrease in the amount of vitamin C in the plasma precedes any decrease in the amount of ascorbic acid in the white blood cell-platelet layer. When vitamin C is absent from the plasma the subject can be considered to be in a state of vitamin C subnutrition. When the vitamin is absent from the white blood cell-platelet layer the subject is approaching the clinical state of scurvy.

Immunological tests

Materials for the various immunological tests were obtained each week or, in certain instances, every second week.

Determination of the capacity of the nasal secretions to inactivate influenza virus

Nasal secretions were obtained by inserting pledgets of dry cotton into the middle meatus of the nose. After the cotton had become well soaked, the clear fluid was expressed. This material was stored at 4° C. and each specimen was subjected to the same period of storage before testing. The virus was obtained from infected mouse lung which had been ground with alundum and, after light centrifugation, diluted 1:500 with 10 per cent normal horse serum in physiological sodium chloride solution. Serial, two-fold dilutions of the nasal secretions in physiological sodium chloride solution were made and to 0.15 cc. of each dilution an equal volume of a suspension of the PR8 (10) strain of type A epidemic influenza virus, containing 6,000 lethal doses, was added. The nasal secretions-virus mixtures were incubated at 37° C. for 30 minutes and then 0.05 cc. of each mixture (containing 1,000 lethal doses of virus) was given intranasally to each of three C.F.W. Swiss mice lightly anesthetized with ether. The mice were observed for 10 days, all deaths were recorded daily, and all survivors were sacrificed and autopsied. The end point of the titration was that final dilution of nasal secretions which protected 50 per cent of the mice from death in 10 days (11). Since these tests were made at weekly intervals, the potency of the virus was determined each week to permit a standardization of the results.

Determination of influenza virus neutralizing antibody in blood serum

These tests were performed in exactly the same manner as those with nasal secretions except that double volumes of diluted serum and virus suspension were placed in the mixture.

A single, standard serum was included in most tests for influenza virus inactivating substance in nasal secretions and influenza virus neutralizing antibody in blood serum. The uniformity of titer as shown in Figure 1 indicates that the results obtained in different tests are strictly comparable since the range of variability fell within one dilution.

Determination of lysozyme in nasal secretions

Serial, two-fold dilutions of nasal secretions were made in physiological sodium chloride solution and 0.5 cc. of each dilution was added to 0.5 cc. of an 18-hour culture of the susceptible microorganism in pneumococcus broth. The mixtures were incubated for one hour at 37° C. The end point was taken as the highest initial dilution of nasal secretions which produced complete lysis of the bacteria.

To ensure uniformity of the bacterial suspensions, tubes containing 8 cc. of broth were seeded with 0.2 cc. of the culture which had been used the previous week and subsequently stored at 4° C. The microorganism employed was a gram positive coccus which produced alpha hemolysis, was not soluble in bile and did not ferment inulin.

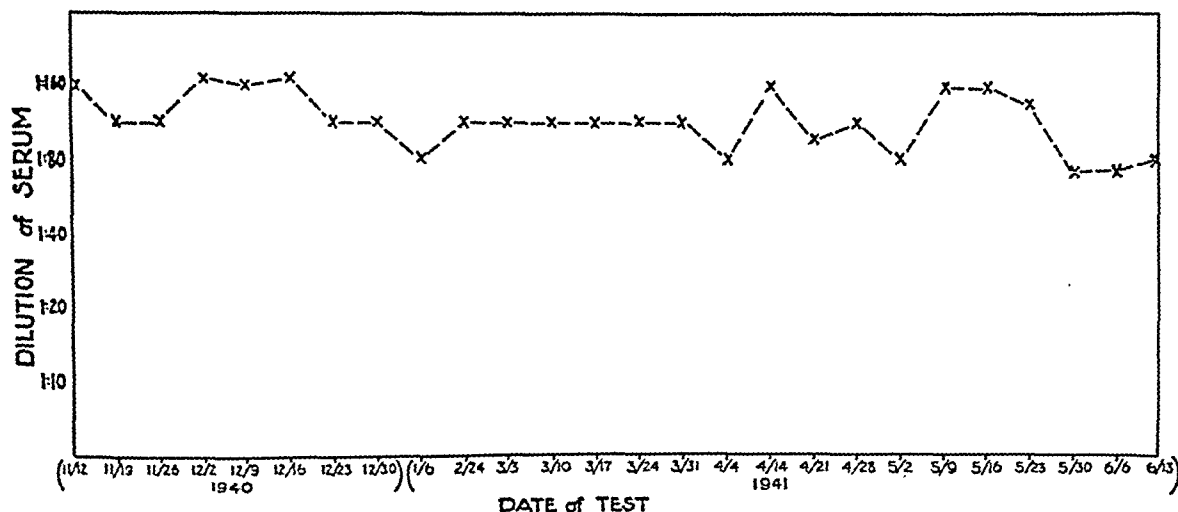


FIG. 1. TITERS OF INFLUENZA VIRUS NEUTRALIZING ANTIBODY AS DETERMINED BY REPEATED TESTS OF A SINGLE, STANDARD SERUM

The substance which was being measured in these tests had the properties of lysozyme (12) since the nasal secretions could be heated at 100° C. for 30 minutes at pH 4.5 and when restored to pH 7 would still cause lysis of the test organism, although the potency was reduced by 50 per cent. Of note was the observation that this treatment completely destroyed the ability of the nasal secretions to inactivate influenza virus.

Determination of complement in blood serum

Blood was drawn into partial vacuum venules² and stored at 4° C. for 30 hours, at which time the serum was removed and titrated. The serum was diluted 1:10, 1:20, 1:30, 1:40, 1:60, 1:80, 1:100, 1:120 and 1:160 in physiological sodium chloride solution. Five-tenths cc. of sensitized sheep red blood cells was added to 0.5 cc. of each dilution of serum and the mixtures incubated for one hour at 37° C. Two end points were read: first, the highest initial dilution of serum producing complete hemolysis and, second, the highest initial dilution of serum producing the least detectable trace of hemolysis, as judged by inspecting the supernate following centrifugation. Control tests consisting of 0.5 cc. of each dilution of the patient's serum, 0.25 cc. of unsensitized sheep red blood cells and 0.25 cc. of physiological sodium chloride solution were always included.

The same lot of amboceptor served as a source of hemolysin for all tests and 0.25 cc. was employed to sensitize 0.25 cc. of the sheep red blood cells. The latter were washed with physiological sodium chloride solution before use.

Determination of the phagocytic index

Approximately 0.75 cc. of the patient's blood was drawn into 0.1 cc. of 0.05 per cent heparin in physiological sodium chloride solution and the mixture was kept at

² Kimble Glass Co., Vineland, N. J.

37° C. for one hour. One-tenth cc. of the heparinized blood and 0.1 cc. of a 1:10 dilution in sterile meat infusion broth (pH 7.8) of an 18-hour culture of pneumococci in pneumococcus broth were then mixed in a 9 × 135 mm. test tube. The tubes were then sealed in a gas-oxygen flame and quickly placed upright in a water bath at 37° C. for 5 minutes. The tubes were then kept for one hour at 37° C. in a device which rotated them end over end 35 times per minute. A drop of the blood-pneumococcus mixture was then spread on a glass slide and stained with Wright's stain. The first 100 polymorphonuclear neutrophilic leukocytes encountered were examined for the presence of engulfed pneumococci. The phagocytic index was taken as the total number of intracellular diplococci divided by 100. The phagocytic index for type one pneumococci, strain S.V.1, and for type two pneumococci, strain D39, was determined each week as a rule.

The stock cultures of the two strains of pneumococci used in the tests were continuously incubated at 37° C. and were subcultured every 24 hours by adding 0.1 cc. of culture to 8 cc. of pneumococcus broth containing 0.1 cc. of normal rabbit blood. Each strain was passed at weekly intervals to a mouse by the intraperitoneal route and recovered from the blood of the heart 3 days before use in the tests. Both strains remained of uniform virulence so that a 10⁻⁶ dilution of culture was fatal for mice in approximately 24 hours. The bacterial colonies were always smooth.

A total leukocyte count of each sample of heparinized blood was made but there was no discernible relationship between the total leukocyte count and the phagocytic index. In no instance was a frank leukocytosis encountered.

CONTROL OBSERVATIONS IN NORMAL SUBJECTS

Certain observations served as controls for the studies in the patients on vitamin deficient diets.

(a) In order to evaluate the significance of changes in titer in the test subjects, it was necessary to obtain parallel determinations of the same nature in normal individuals. Four persons from the laboratory were selected because they habitually consumed entirely adequate, average diets. Over a period of 34 weeks, at weekly intervals, tests for the influenza virus inactivating substance and lysozyme content of nasal secretions were conducted and monthly samples of serum were obtained for titration of neutralizing antibody to influenza virus (Figure 2). These materials were tested each week, together with similar material from all patients under investigation at the time. It was found that the results of each test in each individual tended, despite distinct variations, to remain at a characteristic high, intermediate or low level.

Each of the four subjects while under observation suffered his usual number of common colds. The presence of these infections was in no way reflected in the results of the tests nor was there any obvious relation between the immunological activity of the materials tested and the occurrence of the colds.

A single observation of great interest was made in Subject R. M. In the 26th week, when the subject was in the period of invasion of measles, no influenza virus inactivating substance was detected in the nasal secretions, although 1 week previously and 2 weeks later it was present in high titer.

(b) The phagocytic indices of the blood of three normal persons against pneumococci were determined each week. These tests were followed through periods of 8 weeks, 7 months and 8 months, respectively (Table III). Under the conditions of study, considerable fluctuation in the results was noted but no indication that the variations were due to modifications of bacterial virulence was obtained. The fluctuations were never uniform in all tests done at a given time but rather appeared to represent individual variations. On several occasions the accuracy of the technique was checked by treating two portions of the same sample of heparinized blood as separate specimens throughout the test. The resultant phagocytic indices were essentially the same. The conclusion was reached that the variations seen were related principally to unknown factors in the

TABLE III
Phagocytic indices of blood from three normal persons for type one and type two pneumococci

Date	Subject A.E.F.		Subject S.M.		Subject E.M.	
	Type one	Type two	Type one	Type two	Type one	Type two
October 8, 1940		5.06				
October 10, 1940				1.22		
October 15, 1940		0.05				
October 16, 1940		0.11				
October 17, 1940				0.29		
October 22, 1940		1.10				
October 24, 1940				0.44		
October 29, 1940		3.01				
October 31, 1940				0.29		
November 5, 1940	0.04	7.05				
November 7, 1940			0.02	0.19		
November 14, 1940	0.05	0.29	0.00	0.18		
November 20, 1940	0.03	0.98	0.03	0.09		
November 27, 1940			0.03	0.15		
November 29, 1940	0.01	0.83				
December 4, 1940	0.02	0.47	0.03	0.10		
December 10, 1940				0.22		
December 17, 1940	1.04	0.91	0.20	0.18		
December 24, 1940	0.24	6.70	0.04	0.32		
December 31, 1940	0.10	0.76	0.04	0.37		
January 7, 1941	0.04	1.61	0.07	0.28		
January 14, 1941	0.15	0.99	0.02	0.37		
January 21, 1941	0.10	1.08				
January 28, 1941	0.02	2.50	0.00	1.04		
February 4, 1941	0.10	0.21	0.05	0.04		
February 18, 1941	0.03	0.86				
February 25, 1941	0.06	0.90				
March 4, 1941	0.02	0.67	0.04	0.62		
March 11, 1941	0.13	0.34	0.01	0.17		
March 18, 1941	0.17	1.97	0.01	0.63		
March 25, 1941	0.16	1.09	0.14	0.83		
April 1, 1941	0.50	3.26	0.13	0.32		
April 8, 1941	0.03	0.37	0.34	2.14		
April 15, 1941	1.20	1.83	0.05	0.36		
April 22, 1941	0.82	4.44			0.26	3.26
April 29, 1941	0.49	0.81			0.93	0.54
May 6, 1941	0.75	1.32				
May 13, 1941	0.40	0.88			0.58	0.84
May 20, 1941	0.69	1.32			0.55	0.53
May 27, 1941	0.10	3.70				
June 3, 1941	0.28	0.50			0.15	0.23
June 10, 1941	0.26	2.70			2.87	0.19

blood itself which influenced the phagocytic activity.

EXPERIMENTAL OBSERVATIONS

(a) *The influence of changes in the blood plasma levels of vitamin A and vitamin C upon immunological phenomena*

Immunological studies were made at regular intervals on each of two patients who were under approximately the same experimental conditions. In the course of these studies definite changes were produced in the plasma levels of vitamin A and vitamin C. In addition, tests were made upon

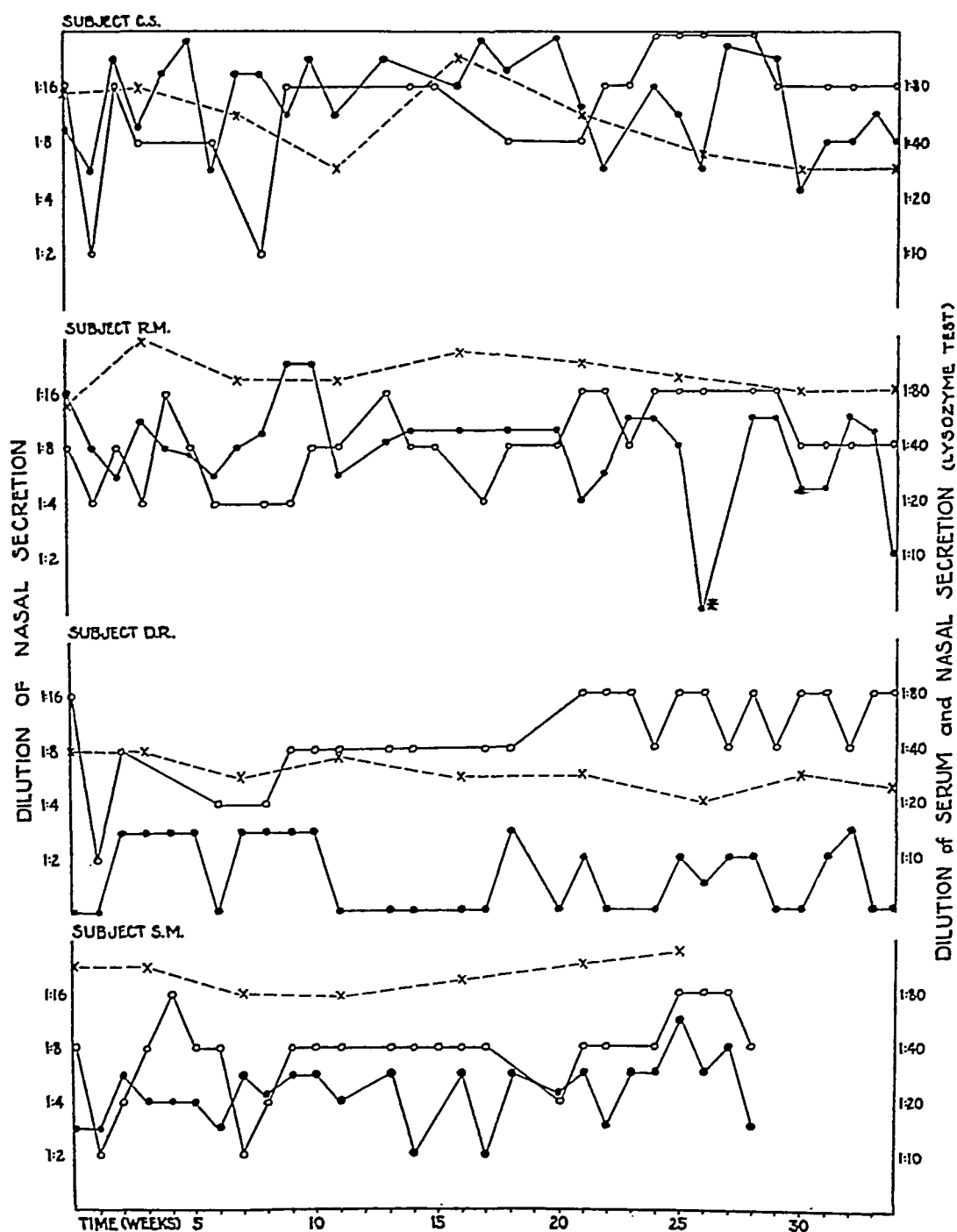


FIG. 2. RESULTS OF TESTS FOR INFLUENZA VIRUS INACTIVATING SUBSTANCE IN NASAL SECRETIONS (●—●), INFLUENZA VIRUS NEUTRALIZING ANTIBODY IN BLOOD SERUM (X—X) AND LYSOZYME IN NASAL SECRETIONS (○—○) OF FOUR NORMAL SUBJECTS AS DETERMINED AT WEEKLY INTERVALS

* Subject in the period of invasion of measles.

materials obtained from each patient before and several hours after the administration of a dose of cod liver oil concentrate sufficient to cause a marked rise in the plasma level of vitamin A.

W. W., a 50-year-old white male, was admitted to the hospital on September 6, 1940. He had lost 35 pounds of weight during the preceding year and had suffered from nervousness and tremors. He was undernourished and had generalized arteriosclerosis.

A high caloric diet was given for 8 days at the end of which time analysis of the blood plasma revealed 0.5 mgm. per cent of vitamin C, 88 U.S.P. units of vitamin A per 100 cc. and 0.13 mgm. per cent of carotene.

On September 17, a 90-day period of observation was begun. The patient was placed on the vitamin A deficient diet supplemented daily with 200 mgm. ascorbic acid, 10 mgm. thiamine chloride, 50 mgm. nicotinic acid, 15 grams dried brewers' yeast and 1.2 grams ferrous sulphate. This regime was continued for 32 days during which time the plasma levels of vitamin A ranged between 75 and 88 U.S.P. units per 100 cc. while the plasma carotene fell progressively to a low level of 0.075 mgm. per cent. By the 10th day, as a result of the administration of vitamin C, the plasma level had risen to 1.87 mgm. per cent and the concentration in the white blood cell-platelet layer was 24 mgm. per 100 grams.

The patient was discharged from the hospital on the 32nd day with instructions to eat a regular diet supplemented daily with 300,000 U.S.P. units of vitamin A in the form of a cod liver oil concentrate. The other dietary supplements were discontinued. The patient returned weekly for further observations. This regime was continued for the final 58 days of the study. As a result of the large intake of vitamin A, the plasma level increased to 143 U.S.P. units per 100 cc. by the 44th day at which time the daily dose of vitamin A was reduced to 100,000 U.S.P. units. By the 58th day, the plasma vitamin A content had decreased to 75 U.S.P. units per 100 cc. On the 82nd day, the patient was given a large dose of cod liver oil (300,000 U.S.P. units of vitamin A). This raised the plasma level of vitamin A from an initial value of 88 to a value of 393 U.S.P. units per 100 cc. in 5 hours. After this the usual daily dose of 100,000 U.S.P. units of vitamin A was resumed. The diet probably contained inadequate amounts of vitamin C because the plasma level decreased progressively to 0.21 mgm. per cent. On the 83rd day, he was again placed on 200 mgm. vitamin C daily and by the 90th day, the plasma level had risen to 1.73 mgm. per cent.

During the studies the patient gained approximately 10 pounds in weight.

T. O., a 60-year-old white male, was admitted to the hospital on September 17, 1940. His chief complaints were abdominal pain for 6 months, loss of 40 pounds of weight during the preceding year and weakness for 6 months. Although he had consumed no liquor for 2½ years, he had previously been a heavy drinker. His diet had been inadequate for 2 years, consisting mostly of

oatmeal and some sort of stew. He took no milk, fruit or vegetables. He suffered frequently from the common cold. The patient weighed 146 pounds and did not appear ill. The skin was slightly icteric. The liver was enlarged, firm and tender and the abdominal veins were dilated. The urine contained bile and urobilin. The icteric index was 21. Red blood cells numbered 5.8 million per cubic millimeter and the blood contained 13 grams hemoglobin per 100 cc. The plasma vitamin A was 25 U.S.P. units per 100 cc. and the plasma carotene was 0.06 mgm. per cent. No vitamin C was detected in the plasma. A diagnosis of cirrhosis of the liver was made.

Due to the low level of vitamin A in the plasma, this patient provided an opportunity to study the immunological phenomena while the plasma vitamin A was kept at a low level and then to observe the possible effects of the administration of cod liver oil in amounts sufficient to raise the plasma level of vitamin A to normal.

A 175-day period of observation was begun on September 24 when the patient was placed on the vitamin A deficient diet supplemented daily with 20 mgm. thiamine chloride, 200 mgm. nicotinic acid and 30 grams dried brewers' yeast. This vitamin A deficient regime was continued for 26 days during which time the plasma level of vitamin A was never above 45 U.S.P. units per 100 cc. and the plasma level of carotene was never above 0.080 mgm. per cent. At the beginning of this 26-day period, there was still no vitamin C in the plasma and the white blood cell-platelet layer contained only 5 mgm. per 100 grams. One thousand mgm. of vitamin C were given daily from the 2nd to the 6th day, at which time the plasma content of vitamin C had risen to 1.05 mgm. per cent and the concentration in the white blood cell-platelet layer was 25 mgm. per 100 grams. From the 6th to the 26th day, the patient received 200 mgm. vitamin C daily and the plasma level of vitamin C remained within the normal range.

From the 26th to the 115th day, the vitamin A deficient diet, the daily supplements of the vitamin B complex and 200 mgm. of vitamin C daily were continued as before but adequate amounts of vitamin A in the form of a cod liver oil concentrate were given daily. From the 26th to the 31st day, 300,000 U.S.P. units of vitamin A were given daily. By the 29th day, the plasma level of vitamin A had risen to 118 U.S.P. units per 100 cc. but the plasma carotene level was still low, 0.050 mgm. per cent. Because of diarrhea, the cod liver oil was discontinued on the 31st day. From the 45th to the 73rd day, 100,000 U.S.P. units of vitamin A were given daily and the plasma levels varied from 75 to 97 U.S.P. units per 100 cc. From the 73rd to the 115th day, the daily dose of vitamin A was increased to 200,000 U.S.P. units and during this period the plasma content varied from 75 to 157 U.S.P. units per 100 cc. Meanwhile, the plasma levels of carotene progressively decreased to 0.015 mgm. per cent, probably because of the absence of carotene-containing foods from the diet. From the 26th to the 115th day, the plasma and white blood cell-platelet levels of vitamin C remained within the normal range due to

the administration of adequate amounts of the vitamin daily.

From the 115th to the 175th day, the patient continued to receive 200,000 U.S.P. units of vitamin A daily plus the vitamin B complex but all sources of vitamin C were removed from the diet and no supplements of vitamin C were given. The plasma levels of vitamin A and carotene remained at their former values during this final period of study but the plasma level of vitamin C gradually fell to zero by the 175th day.

On the 112th day, a dose of cod liver oil containing 300,000 U.S.P. units of vitamin A was given and the plasma level of the vitamin rose from a fasting value of 153 to 815 U.S.P. units per 100 cc. in 5 hours and then fell to 157 U.S.P. units per 100 cc. in the next 20 hours.

During the studies the patient suffered several attacks of the common cold; two of them occurred while he was taking vitamin A and vitamin C. The jaundice and abdominal pain gradually disappeared. The patient gained approximately 25 pounds in weight.

Although marked changes were produced and maintained in the plasma levels of vitamin A and vitamin C by the addition or removal of these substances from the diet, noteworthy alterations in the results of the immunological tests were not observed (Figures 3 and 4). Furthermore, abrupt rises in the plasma content of vitamin A were induced by the administration of a large dose of this vitamin. Materials for the immunological tests were obtained from each patient just before the administration of the vitamin A, at a time when the plasma levels of this vitamin were normal, and again 5 hours later when the levels were 393 and 815 U.S.P. units per 100 cc., respectively. In neither instance were the results of the immunological tests appreciably influenced by the abrupt increase in the vitamin A content of the plasma.

(b) The influence of vitamin C deficiency upon immunological phenomena

Immunological studies were made at regular intervals in each of two patients who were kept on the regime deficient in vitamin C until they were thoroughly depleted of the vitamin and who were then given amounts of vitamin C adequate to restore the plasma and white blood cell-platelet levels to normal.

J. M., a 54-year-old white male, was admitted to the hospital on October 31, 1940. His chief complaint was pain in the left upper portion of the chest anteriorly and in the left shoulder and arm. These pains were due to a mild arthritis of the dorsal spine and left shoulder joint.

The patient was in good condition and well nourished. The plasma vitamin C was 0.21 mgm. per cent on November 27.

A 130-day period of observation was begun on November 29, when the patient was placed on the vitamin C deficient diet supplemented daily with 20 mgm. thiamine chloride, 100 mgm. nicotinic acid, 2 mgm. riboflavin, 30 grams dried brewers' yeast and 100,000 U.S.P. units of vitamin A in the form of a cod liver oil concentrate. The plasma level of vitamin C fell gradually and reached zero by the 34th day, at which time the concentration of ascorbic acid in the white blood cell-platelet layer was 10 mgm. per 100 grams. Vitamin C was absent from the plasma from the 34th to the 97th day and during this interval of 63 days the amount of ascorbic acid in the white blood cell-platelet layer varied from 3 to 9 mgm. per 100 grams.

From the 97th day to the end of the study (a period of 33 days), 200 mgm. of vitamin C were given by mouth daily but the regime was otherwise unchanged. On the 102nd day, no vitamin C was present in the plasma and the amount of ascorbic acid in the white blood cell-platelet layer was only 5 mgm. per 100 grams. Thereafter, the plasma level of vitamin C rose slowly to 0.88 mgm. per cent by the 130th day, at which time the concentration in the white blood cell-platelet layer was 20 mgm. per 100 grams.

Throughout the study the patient received large daily doses of vitamin A. This resulted in a rise in the plasma level of vitamin A from an initial value of 105 U.S.P. units per 100 cc. to values which were usually above 150 and frequently exceeded 300 U.S.P. units per 100 cc. The diet was deficient in foods containing carotene so that, in spite of the high levels of vitamin A in the plasma and the large daily doses of cod liver oil, the plasma carotene level fell from an original value of 0.135 mgm. per cent to 0.040 mgm. per cent on the 88th day and was only 0.065 mgm. per cent on the 130th day.

Signs or symptoms of scurvy did not appear although vitamin C was absent from the plasma for 63 consecutive days and the amount of ascorbic acid in the white blood cell-platelet layer was reduced to 3 mgm. per 100 grams. This observation was not unexpected since Crandon *et al.* (13) and Ralli and Sherry (14) have also reported instances in which vitamin C has been absent from the plasma for considerable periods of time without clinical evidence of scurvy. In Crandon's report (13), a diet deficient in vitamin C was continued for 140 days before the first clinical signs of scurvy appeared. In the present case there was little doubt that the patient's tissues had been thoroughly depleted of vitamin C as 5900 mgm. of the vitamin were required to resaturate the plasma and white blood cell-platelet layer.

The patient gained approximately 33 pounds in weight during the study, thus emphasizing the adequacy of the diet in other respects.

The depletion of vitamin C in this patient and the subsequent saturation of the tissues by the ad-

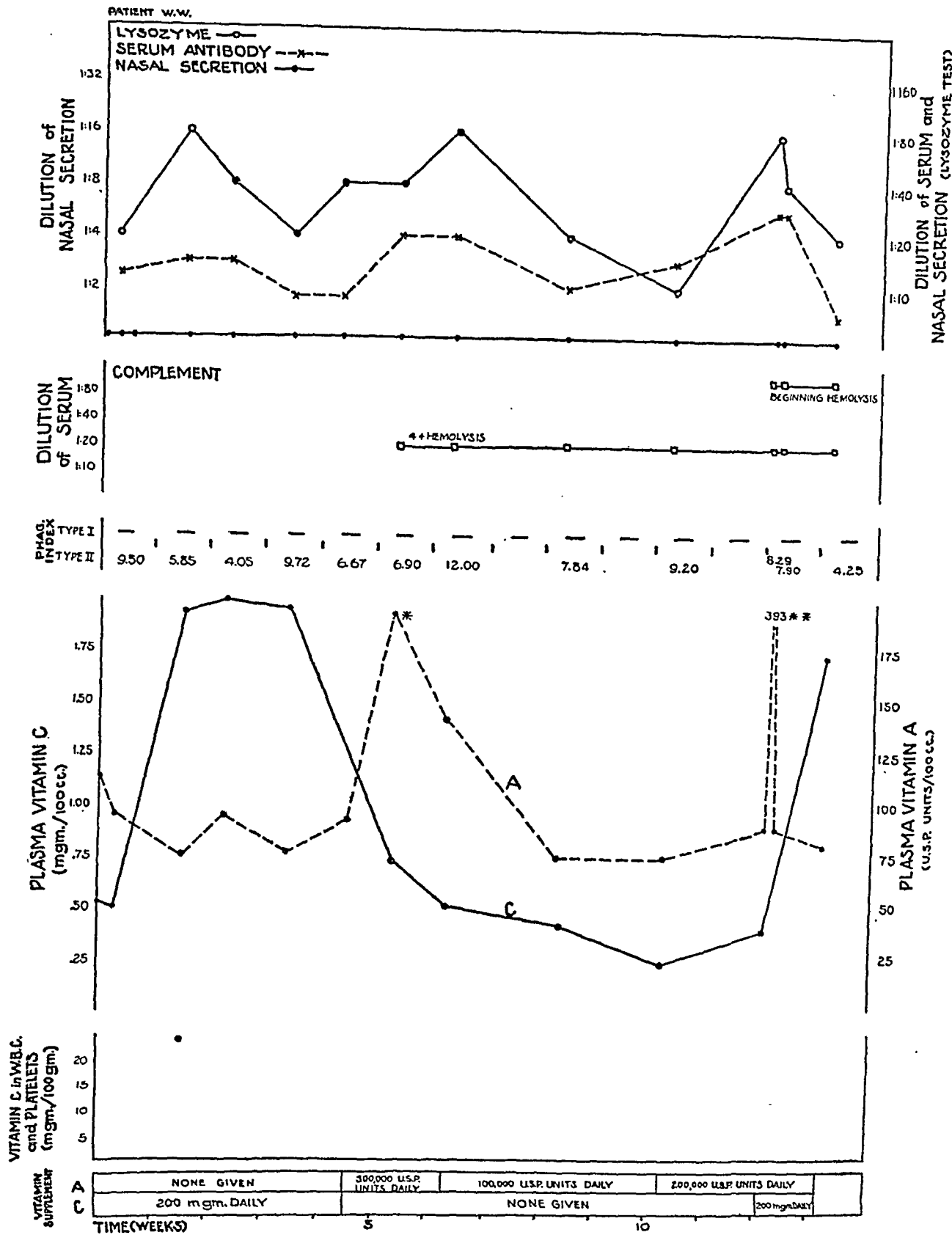


FIG. 3. RESULTS OF IMMUNOLOGICAL TESTS IN PATIENT W. W.

* This determination was made 2 hours after the patient had taken cod liver oil and does not represent the fasting level of vitamin A.

** This figure is the level of vitamin A 5 hours after the administration of 300,000 U.S.P. units of vitamin A. The immunological tests were performed on materials obtained immediately previous to and 5 hours after the administration of the vitamin A.

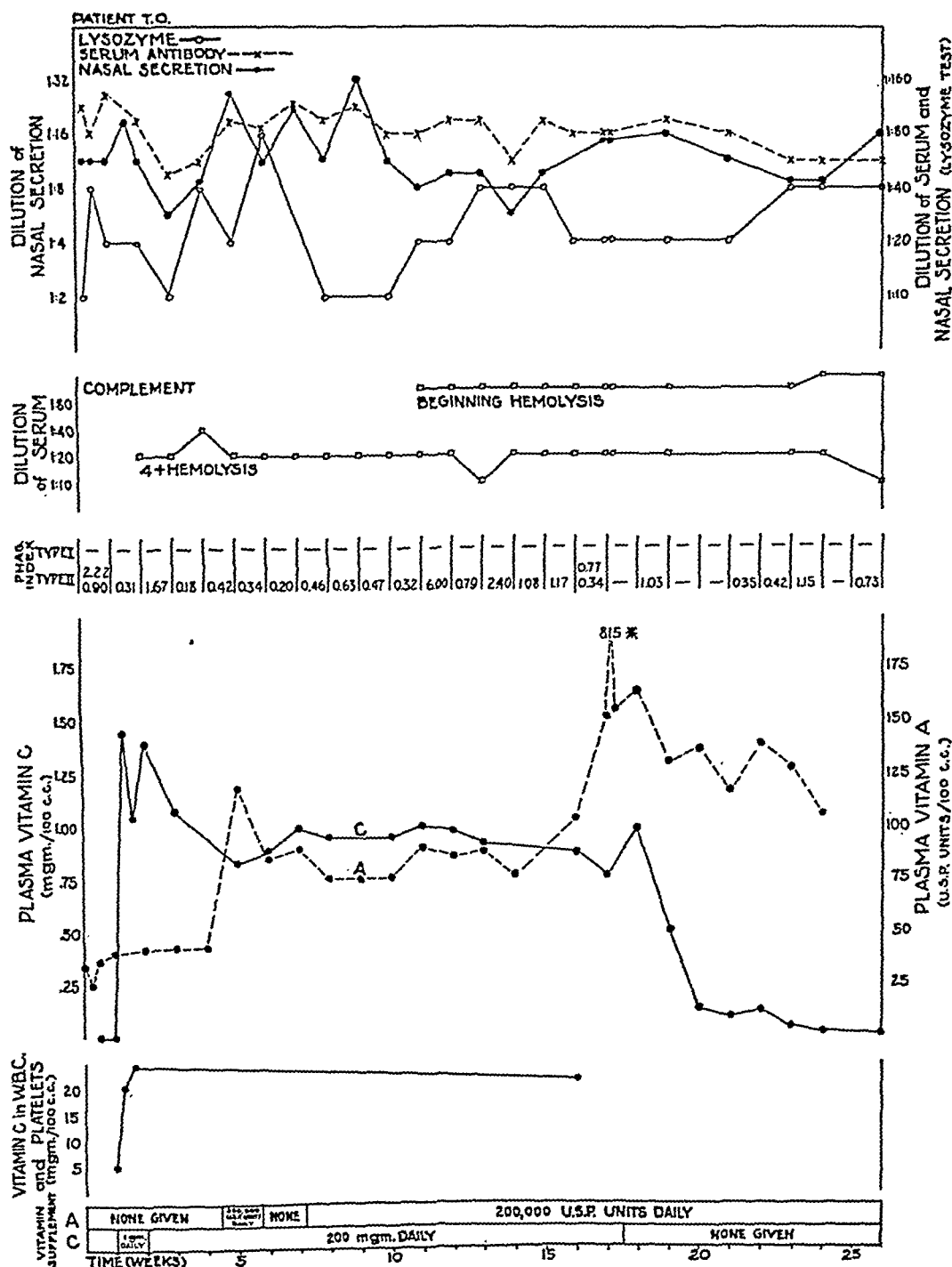


FIG. 4. RESULTS OF IMMUNOLOGICAL TESTS IN PATIENT T. O.

* This figure is the level of vitamin A 5 hours after the administration of 300,000 U.S.P. units of vitamin A. The immunological tests were performed on materials obtained immediately previous to and 5 hours after the administration of the vitamin A.

ministration of ascorbic acid produced no significant alterations in the results of the immunological tests (Figure 5). During the final 5 weeks of the study, when the patient was receiving 200 mgm. of vitamin C daily and the plasma levels were progressively increasing, determinations of the influenza virus inactivating substance in the nasal secretions yielded one result definitely below the average and two results clearly higher than the average. These variations appear to have little significance because fluctuations of equal or greater magnitude were frequently demonstrated in the titers for this substance in nasal secretions obtained from normal persons (Figure 2). Five of the final seven phagocytic indices for type two pneumococci exceeded the upper limits of the values previously observed. This increase in the phagocytic power of the blood was probably unrelated either to the vitamin C deficiency or to the subsequent saturation of the tissues with ascorbic acid because the indices were significantly increased before the patient was given vitamin C and there was no comparable increase in the indices for type one pneumococci. Furthermore, it was demonstrated by means of the mouse protection test that serum obtained during the 21st week of observation, when the phagocytic index for type two pneumococci was 7.52, contained type specific antibodies for type two pneumococci, whereas none was present in serum obtained during the 11th week. The test was performed by testing each specimen of serum with three dilutions of culture of pneumococci, namely, 10^{-4} , 10^{-5} , 10^{-6} . Three mice were injected with each mixture of serum and pneumococci. All mice receiving mixtures of pneumococci and serum taken in the 11th week died. All mice receiving mixtures of serum taken in the 21st week and pneumococci in a dilution of 10^{-4} and 10^{-5} also died but two of the three animals receiving the 10^{-6} dilution of pneumococci (10 lethal doses per animal) survived. The elaboration of type specific antibody was most probably the result of an actual stimulus by the specific antigen.

Of special interest was the observation that the amount of complement in the blood serum as measured by the technique described above was not decreased in this patient during the period of vitamin C deficiency. This confirms the report of Candon *et al.* (13) who found no decrease in serum

complement despite a severe depletion of vitamin C. Others (15, 16) have stated that a decrease in complement parallels a deficiency in vitamin C.

L. N., a 34-year-old negro male with a history of alcoholism, was admitted to the hospital on April 15, 1941. He had an acute alcoholic gastritis but it subsided in a few days. He was in a good state of nutrition.

A 47-day period of observation was begun on April 24 when the patient was placed on the vitamin C deficient diet with daily supplements of 10 mgm. thiamine chloride, 100 mgm. nicotinic acid and 30 grams of dried brewers' yeast. On the 5th day, daily supplements of 100,000 U.S.P. units of vitamin A in the form of a cod liver oil concentrate were begun. This regime was continued for 40 days and during this period the plasma level of vitamin C remained at zero and the concentration of ascorbic acid in the white blood cell-platelet layer varied between 6 and 9 mgm. per 100 grams.

On the 40th day, daily supplements of 500 mgm. of vitamin C were added. By the 47th day, the plasma vitamin C had risen to 1.25 mgm. per cent and the level in the white blood cell-platelet layer had increased to a value of 33 mgm. per 100 grams.

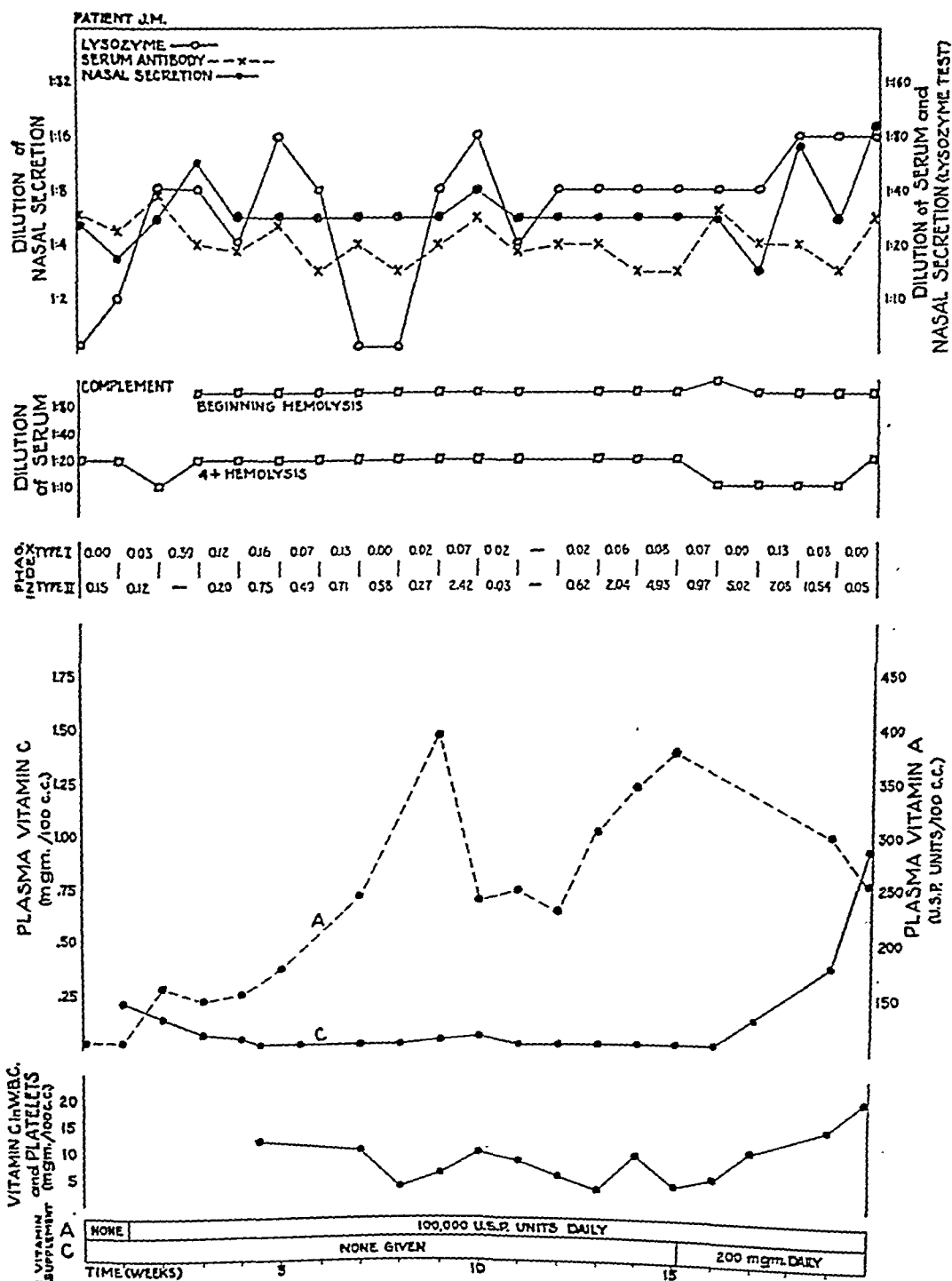
The amount of vitamin A in the plasma ranged from 78 to 195 U.S.P. units per 100 cc. during the 47 days of observation while the amount of carotene in the plasma decreased from an initial value of 0.080 mgm. per cent to a final value of 0.065 mgm. per cent.

On the 27th day, the patient developed an acute pharyngitis with cervical lymphadenopathy and fever of 102.6° F. Beta hemolytic streptococci, Lancefield group A, were isolated from the pharynx. The course of the illness was not unusual despite the deficiency in vitamin C and the patient was well by the 33rd day. Only symptomatic treatment was used.

The deficiency of vitamin C in this patient was severe. This was shown by the fact that the amount of vitamin C required to saturate the tissues (3500 mgm.) was approximately the amount required to saturate the tissues of patients suffering from scurvy (17).

The patient gained 7 pounds in weight during the study.

At no time during the period of observation were the titers of lysozyme in the nasal secretions, the amounts of complement in the blood serum or the phagocytic indices of the blood for pneumococci altered to a significant degree (Figure 6). The fact that the phagocytic indices were not influenced either by the deprivation of vitamin C or by the subsequent saturation of the tissues with the vitamin supports the impression that the increase in the phagocytic indices for type two pneumococci observed in the previous patient (*J. M.*) subjected to prolonged deprivation of vitamin C was unrelated either to the deficiency or to the administration of the vitamin.



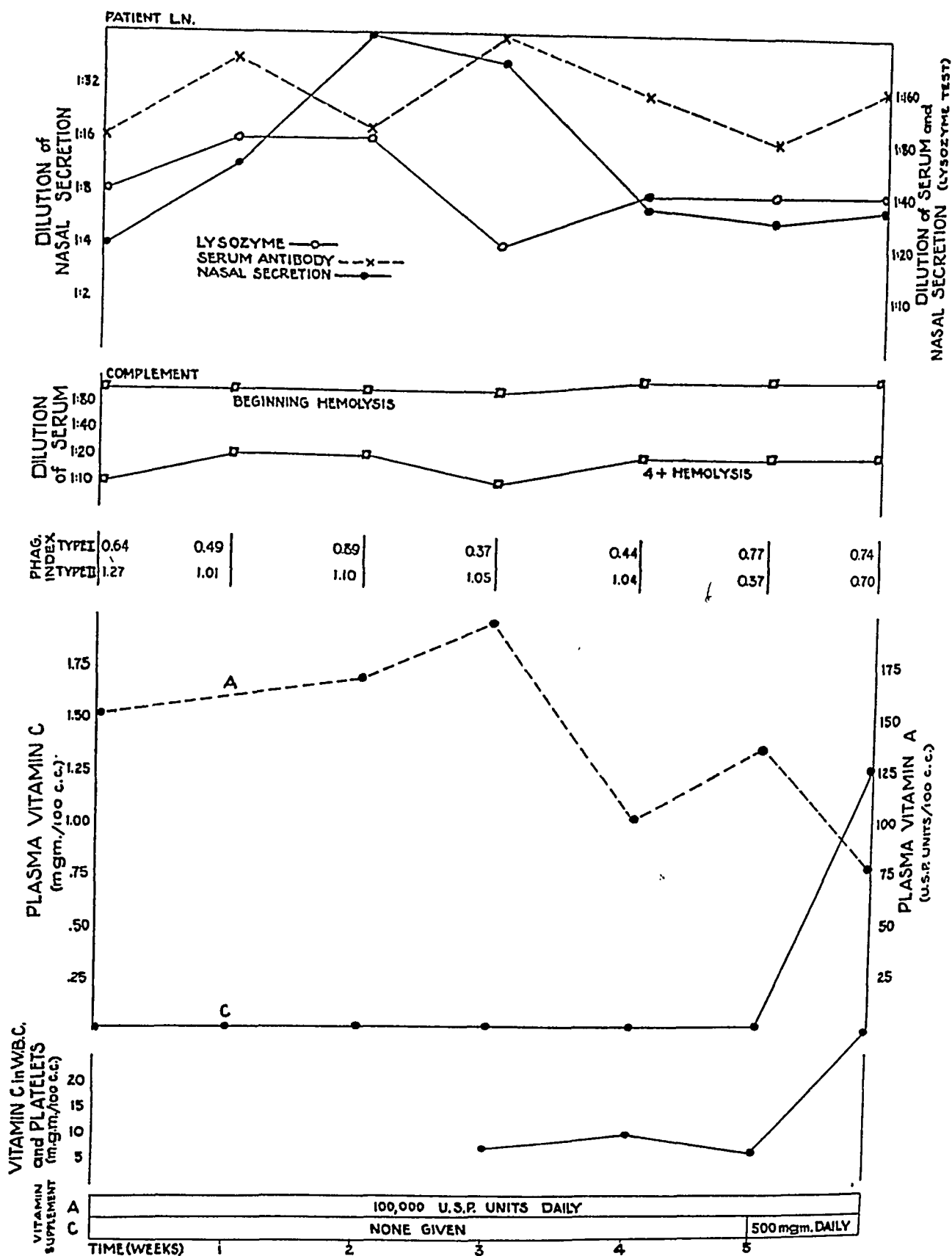


FIG. 6. RESULTS OF IMMUNOLOGICAL TESTS IN PATIENT L. N.

On the other hand, the variations in the titer of influenza virus inactivating substance in the nasal secretions and of influenza virus neutralizing antibody in the serum were of interest. The titers increased during the first 3 weeks of observation, remained at a high level in the 4th week and fell to lower levels in the 5th and 6th weeks. These observations were not readily explained. That they were related either to the depletion of vitamin C or to the saturation of the tissues with the vitamin is not clear since the titers both rose and fell during the period when the amount of vitamin C in the blood was extremely small and were not influenced appreciably by the administration of 500 mgm. of ascorbic acid daily for one week. The amount of vitamin A in the plasma was within normal limits during the entire period of study. The attack of pharyngitis did not occur until the 4th week, at which time the titers were decreasing. It could be suggested that the increase in virus neutralizing titers was due to specific infection with influenza virus. There was no clinical evidence that such infection had occurred.

(c) The influence of a diet deficient in vitamin A upon immunological phenomena

Immunological studies were made at regular intervals on a patient who was kept on a diet deficient in vitamin A for 17 weeks and who was then given large amounts of vitamin A daily during the final 2 weeks of study. In addition, materials for the various immunological tests were obtained before and 3 hours after the initial administration of vitamin A immediately subsequent to the period of deprivation in order to test the immunological phenomena at a time when the plasma was flooded with the vitamin.

A. M., a 33-year-old white male, was admitted to the hospital on December 23, 1940. Except for an ankylosing arthritis of the spine and hip joints, he was normal and well nourished. On admission, the vitamin C in the plasma was 0.09 mgm. per cent; the vitamin A in the plasma was 135 U.S.P. units per 100 cc.; the carotene in the plasma was 0.110 mgm. per cent.

A 126-day period of study was begun on January 28, when the patient was placed on the diet deficient in vitamin A with daily supplements of 10 mgm. thiamine chloride, 200 mgm. nicotinic acid, 30 grams of dried brewers' yeast and 200 mgm. vitamin C. Although this diet which was deficient in vitamin A was continued for 112 days,

the level of vitamin A in the plasma never fell below the values observed in normal subjects.

From the 113th to the 126th day, a daily supplement of 100,000 U.S.P. units of vitamin A in the form of a cod liver oil concentrate was added but the fasting levels of vitamin A in the plasma determined at weekly intervals were not affected appreciably. However, the level of vitamin A in the plasma determined subsequent to the period of deprivation and 3 hours after the initial dose of the vitamin did rise from a fasting value of 98 U.S.P. units per 100 cc. to 405 U.S.P. units per 100 cc. Three hours later it fell to a level of 310 U.S.P. units and was back again to a normal value of 123 U.S.P. units after 24 hours.

On the 7th day the daily supplement of vitamin C was reduced to 100 mgm. but, despite the fact that this amount of ascorbic acid is known to be adequate (9), the level of vitamin C in the plasma fell from 1.06 mgm. per cent and by the 70th day was 0.39 mgm. per cent. On the 105th day, the daily supplement of vitamin C was increased to 200 mgm. for 6 days. Although the level of vitamin C in the plasma rose only to a value of 0.43 mgm. per cent, the concentration in the white blood cell-platelet layer was 22 mgm. per 100 grams.

The amount of carotene in the plasma fell progressively from an initial value of 0.095 mgm. per cent to a value of 0.025 mgm. per cent on the 126th day. This was probably due to the absence of carotene from the diet.

The patient remained well and gained 15 pounds in weight during the study. No manifestations of a deficiency in vitamin A were detected.

If the level of vitamin A in the plasma reflects the concentration of the vitamin in the tissues, this patient cannot be considered to have been depleted of vitamin A. The 17-week period on the regime deficient in vitamin A did not appreciably lower the level of the vitamin in the plasma. Furthermore, the response, as measured by the level of vitamin A in the plasma following the administration of 100,000 U.S.P. units of the vitamin subsequent to the period of deprivation, was similar to the response observed in normal subjects after the same dose (8). Granted that a definite deficiency in vitamin A was not produced in this patient, the fact remains that both during a prolonged period of deprivation of the vitamin and during a subsequent period of 2 weeks when the patient was given large doses of vitamin A daily, no noteworthy alterations in the results of the immunological tests were observed (Figure 7). Briefly stated, a period of 17 weeks on a diet deficient in vitamin A had no appreciable influence on the immunological reactions which were measured.

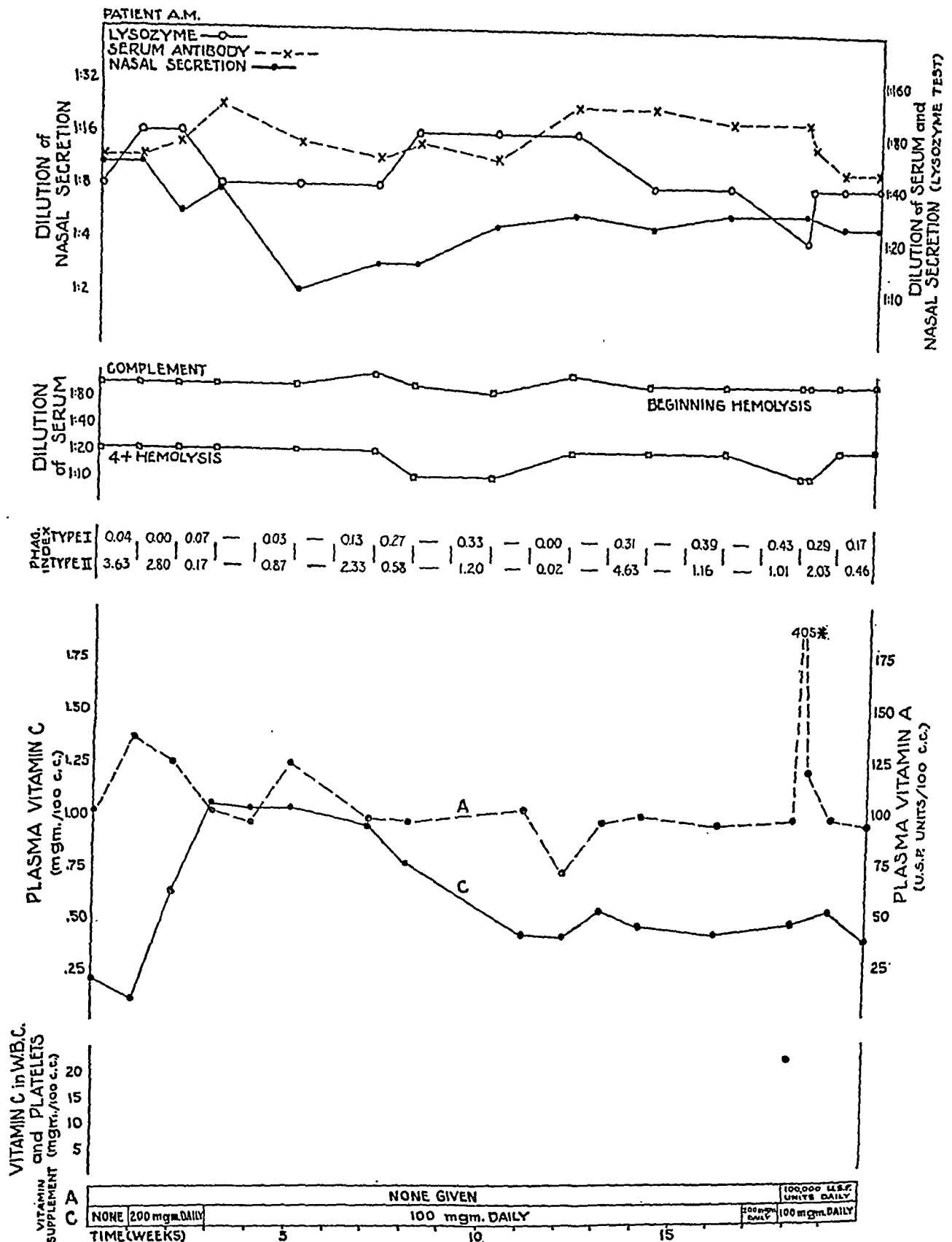


FIG. 7. RESULTS OF IMMUNOLOGICAL TESTS IN PATIENT A. M.

* This figure is the level of vitamin A 3 hours after the administration of 100,000 U.S.P. units of vitamin A. The immunological tests were performed on materials obtained immediately previous to and 3 hours after the administration of the vitamin A.

DISCUSSION

The majority of studies (2) concerning the relation of vitamin deficiencies and resistance to infection are open to certain obvious criticisms. In the human subject most investigations have dealt with nutritional deficiency diseases which are rarely, if ever, attributable to deficiency of a single vitamin. In addition, most studies have only extended over a brief period of time and other dietary factors have not been adequately controlled. Moreover, the immunological examinations have usually considered but one phenomenon. In animal experiments, as for instance those reporting close correlation between complement activity and vitamin C content of the blood (18), the parallel loss in weight due to the cessation of eating has been disregarded. The plan of the present investigation has sought to eliminate these objections. All patients received diets adequate both in calories and essential foodstuffs; in addition, large amounts of vitamins were given daily excepting the specific vitamin, the influence of which was to be determined. The observations extended over relatively long intervals; the shortest period was 6 weeks, while the others lasted from 14 to 26 weeks. Under these conditions, repeated immunological determinations could be made in each phase of dietary modification and errors due to single observations were avoided. The patients were kept in the hospital, the diets were controlled so that all patients gained weight and the daily supplements of vitamin were accurately administered. Control observations of normal subjects on an average diet were similarly conducted. The immunological reactions studied included supposedly physiological or natural phenomena such as complement, lysozyme and pneumococcal activity, together with apparently specific immune reactions to influenza virus. Thus the influence of pronounced and sustained shifts in the concentrations of vitamin A or vitamin C in the blood was more likely to be detected. These two vitamins were selected for study because they have received the most attention in the relation of vitamins to resistance.

Under the experimental conditions described no consistent parallelism between the amount of vitamin A or vitamin C in the blood and the degree of immunological activity has been observed.

Fluctuations in the titers of the various reactions occurred but they varied within narrow limits with relatively few exceptions. Nor were they essentially different from the variations noted in the normal controls. Only two possible correlations were suggested. Examination of Figures 3, 4, 5, 6 and 7 shows that at many points the curve representing the titer of lysozyme in the nasal secretions tends to follow the curve representing the level of vitamin A in the plasma. The parallelism is not consistent, however, and in several instances the two curves obviously diverge. In the two patients subjected to prolonged deprivation of vitamin C and vitamin A, respectively (Figures 5 and 7), another feature was observed. In the periods at the beginning and end of the study when the vitamin levels in the blood were normal a rather constant fluctuation in the titers of lysozyme and virus inactivating substance in the nasal secretions was seen but in the interval of prolonged vitamin deprivation the fluctuations disappeared and a constant level was obtained. The data do not permit of any further comment.

In view of the reported influence of vitamin C upon complement (15, 16), it is interesting to note that in the cases herein reported the complement titrations were rather uniform whether the vitamin C stores were depleted or whether an excess of vitamin C was administered. This is in accord with acute experiments described by Spink *et al.* (19). The results in general have failed to disclose a significant effect of vitamin A or vitamin C upon the phenomena involved in the serological tests.

The immunological tests employed in this study represent reactions that have been found to occur under conditions of health. Whether or not they may serve as reliable indices of resistance to infection is not certain since their rôle in the natural resistance of man has not been clearly determined. Because of the multiplicity of factors that constitute the mechanisms of virulence, susceptibility and host resistance, it is obvious that broad conclusions cannot be drawn from the present findings. However, the tests that were employed were arbitrarily selected because of their diversity and because each represents a biological phenomenon which is either antibacterial or antiviral in its action. They illustrate, therefore, certain

types of immunological reactivity of the patients under conditions of vitamin deficiency and adequacy and furnish information concerning them. Certain additional observations suggest that not only was the immunological reactivity of the patients, as measured by the tests, unimpaired, but that the ability of the patients to resist infection was also intact. L. N. developed a hemolytic streptococcal pharyngitis during the time when he was severely depleted of vitamin C, yet the clinical course of the infection was not remarkable and recovery was complete despite the lack of specific therapy. Furthermore, in this same patient the titers of influenza virus inactivating substance in the nasal secretions and influenza virus neutralizing antibody in the serum tended to increase during the time when he was being depleted of vitamin C and there was no ascorbic acid detectable in the plasma. A. M. was repeatedly exposed to L. N. when he had the pharyngitis, yet A. M. developed no signs of illness despite the fact that he was on the diet deficient in vitamin A. It is to be recalled that J. M. developed type specific antibodies for type two pneumococci in his serum when he was severely depleted of vitamin C. This was presumably due to an infectious stimulus, yet no signs of illness appeared. Only one of the patients contracted severe colds during the period of study and he (T. O.) had two rather severe upper respiratory infections at the time when he was receiving large doses of vitamin A and vitamin C daily.

It was not the purpose of the study to produce the clinical manifestations of a deficiency either of vitamin A or vitamin C. Severe depletion of vitamin C was produced in each of two subjects but a parallel deficiency in vitamin A, as measured by the level of the vitamin in the plasma, was not produced by a diet low in the vitamin in spite of the fact that one patient was kept on such a diet for 17 consecutive weeks. Apparently, the production of a deficiency in vitamin A requires considerably more time than had been allowed. This is probably due to the fact that the stores of vitamin A in the livers of human individuals are considerable (20). Murrill *et al.* (21) kept two normal persons on a diet deficient in vitamin A for 39 and 42 days, respectively, but the plasma levels of the vitamin were not significantly altered. They concluded that the concentration of vitamin

A in the plasma is altered only in extreme cases of vitamin A deficiency.

It was interesting that the level of carotene in the plasma fell significantly in each of the patients on the diet low in vitamin A (and carotene). It is probable that the decrease in the plasma levels of carotene may be taken as evidence of the limited amount of available vitamin A in the diet since carotene is the precursor of vitamin A and is the form in which the vitamin is normally ingested. Murrill *et al.* (21) also noted a decrease in the plasma levels of carotene in their two patients kept on diets low in vitamin A.

SUMMARY

The influence of vitamin A and vitamin C upon a series of immunological phenomena in human individuals has been investigated. Each of five patients was given a diet adequate in all factors but for the specific vitamin, the effect of which was to be determined. The subjects were studied for periods of 14, 26, 20, 6 and 19 weeks, respectively. Determinations of the amount of vitamin A, carotene and vitamin C in the plasma and, in certain instances, measurements of the concentration of vitamin C in the white blood cell-platelet layer were made at weekly or bi-weekly intervals. At similar intervals, observations were made on the following immunological reactions: (1) the capacity of the patient's nasal secretions to inactivate influenza virus; (2) the titer in the patient's blood serum of neutralizing antibodies for influenza virus; (3) the activity of lysozyme in the nasal secretions; (4) the titer of complement in blood serum; and (5) the phagocytic activity for pneumococci of polymorphonuclear neutrophilic leukocytes in heparinized blood.

The results of the various immunological tests were not significantly influenced by the following conditions: (1) marked and prolonged changes in the plasma levels of vitamin A or vitamin C or abrupt rises in the concentration of vitamin A in the plasma (two patients); (2) severe deficiency in vitamin C followed by a period during which the subjects were flooded with the vitamin (two patients); (3) a period of 17 consecutive weeks on a regime deficient in vitamin A followed by a period of 2 weeks during which the subject received large doses of the vitamin daily. Certain

variations in the results of the immunological tests occurred but these alterations were either no more marked than those occurring in normal persons or were adequately explained on some basis other than a deficiency or sufficiency of vitamin A or vitamin C.

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BIBLIOGRAPHY

1. Perla, D., and Marmorston, J., *Natural Resistance and Clinical Medicine*. Little, Brown and Co., Boston, 1941.
- 2a. Clausen, S. W., The influence of nutrition upon resistance to infection. *Phys. Rev.*, 1934, 14, 309.
- b. Robertson, E. C., The vitamins and resistance to infection. *Medicine*, 1934, 13, 123.
- c. Clausen, S. W., *The Pharmacology and Therapeutics of Vitamin A*. The Vitamins, American Medical Association, Chicago, 1939.
- d. Abt, A. F., and Farmer, C. J., *Vitamin C, Pharmacology and Therapeutics*. The Vitamins, American Medical Association, Chicago, 1939.
- 3a. Francis, T., Jr., Inactivation of epidemic influenza virus by nasal secretions of human individuals. *Science*, 1940, 91, 198.
- b. Francis, T., Jr., *The Significance of Nasal Factors in Epidemic Influenza*. Problems and Trends in Virus Research. University of Pennsylvania Press, Philadelphia, 1941, p. 41.
4. Kimble, M. S., The photocolorimetric determination of vitamin A and carotene in human plasma. *J. Lab. and Clin. Med.*, 1939, 24, 1055.
5. Stueck, G. H., Flaum, G., and Ralli, E. P., The serum carotene in diabetic patients. *J. A. M. A.*, 1937, 109, 343.
6. Mindlin, R. L., and Butler, A. M., The determination of ascorbic acid in plasma; a macromethod and micromethod. *J. Biol. Chem.*, 1938, 122, 673.
7. Butler, A. M., and Cushman, M., Distribution of ascorbic acid in the blood and its nutritional significance. *J. Clin. Invest.*, 1940, 19, 459.
- 8a. Ralli, E. P., Bauman, E., and Roberts, L. B., The plasma levels of vitamin A and carotene in normals, in diabetes mellitus and in cirrhosis of the liver. (In press.)
- b. *Idem*, The plasma levels of vitamin A after the ingestion of standard doses: Studies in normal subjects and patients with cirrhosis of the liver. *J. Clin. Invest.*, 1941, 20, 709.
9. Ralli, E. P., Friedman, G. J., and Sherry, S., The vitamin C requirement of man. *J. Clin. Invest.*, 1939, 18, 705.
10. Francis, T., Jr., Transmission of influenza by a filterable virus. *Science*, 1934, 80, 457.
11. Reed, L. J., and Muench, H., A simple method of estimating fifty per cent end points. *Am. J. Hyg.*, 1938, 27, 493.
- 12a. Fleming, A., On a remarkable bacteriolytic element found in tissues and secretions. *Proc. Roy. Soc., London, s.B.*, 1922, 93, 306.
- b. Thompson, R., Lysozyme and its relation to antibacterial properties of various tissues and secretions. *Arch. Path.*, 1940, 30, 1096.
13. Crandon, J. H., Lund, C. C., and Dill, D. B., Experimental human scurvy. *New England J. Med.*, 1940, 223, 353.
14. Ralli, E. P., and Sherry, S., Adult scurvy and the metabolism of vitamin C. *Medicine*, 1941, 20, 251.
15. Ecker, E. E., Pillemer, L., Griffiths, J. J., and Schwartz, W. P., Complement and ascorbic acid in human scurvy. *J. A. M. A.*, 1939, 112, 1449.
16. Chu, Fu-T'ang, and Chow, B. F., Correlation between vitamin C content and complement titer of human blood plasma. *Proc. Soc. Exper. Biol. and Med.*, 1938, 38, 679.
17. Ralli, E. P., and Friedman, G. J., The response to the feeding of cevitamic acid in normal and deficient subjects as measured by a vitamin C excretory test. *Ann. Int. Med.*, 1938, 11, 1996.
18. Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., Ascorbic acid and complement function. *J. Immunol.*, 1938, 34, 19.
19. Spink, W. W., Michelsen, O., and Agnew, S., The relation of ascorbic acid to human complement. *J. Clin. Invest.*, 1941, 20, 434.
20. Ralli, E. P., Papper, E., Paley, K., and Bauman, E., The vitamin A content of the liver in normal and diseased subjects. *Arch. Int. Med.*, 1941, 68, 102.
21. Murrill, W. A., Horton, P. B., Leiberman, E., and Newburgh, L. H., Vitamin A and carotene. II. Vitamin A and carotene metabolism in diabetics and normals. *J. Clin. Invest.*, 1941, 20, 395.

THE INFLUENCE OF DIET ON THE ASCORBIC ACID REQUIREMENT OF PREMATURE INFANTS

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The purpose of this report is to present data, obtained on premature infants, demonstrating that one of the factors determining the need for ascorbic acid is the level of protein intake. It is proposed that this factor plays a rôle in the greater requirement of artificially fed babies, as compared to breast-fed infants, for supplements containing this vitamin.

Long before actual data were available as to the daily total ascorbic acid requirement of the newborn infant, or as to the amount of the vitamin present in human and other milks, it was the common clinical observation that when scurvy occurred in infancy it was in the artificially fed baby. It was assumed that the protection of the breast-fed infant against the disease could be attributed to the antiscorbutic activity of breast milk. This opinion was confirmed by experimental evidence, which has been well summarized in a recent review by Smith (1). The evidence includes the important findings of Selleg and King (2) who showed that the actual amount of ascorbic acid received by an infant ingesting an adequate volume of breast milk from a mother whose diet is adequate in this factor closely approaches the amount which other observers have determined to be the approximate daily requirement of the newborn infant. Determinations of blood plasma ascorbic acid levels by Braestrup (3), Mindlin (4, 5), Snelling (6) and others in babies of various ages, but particularly in the first weeks of life, reveal that these levels are on the average much lower in formula-fed than in breast-fed infants. Moreover, Mindlin (5) demonstrated a definite relationship between the plasma ascorbic acid level of the infant and the amount shown to be present in the mother's milk. However, Mindlin also found (4, 7) that even with supplements of 20 to 75 mgm. of ascorbic acid daily, formula-fed infants had lower levels, determined 16 hours after the last vitamin C dose, than did breast-fed in-

fants receiving no supplements, and Braestrup (3) observed that daily dosages of 20 mgm. of ascorbic acid had a slow effect in raising the plasma levels in babies given boiled cow's milk. These observations are only partially explained on the assumption that the ascorbic acid requirement of the breast-fed and the formula-fed infant is the same, and that the former receives all or part of it in milk whereas the latter, whose feeding is prepared by heating in the presence of air, receives practically none. The supposition that the total daily requirement of infants receiving cow's milk is actually higher than that of infants receiving human milk is more completely consistent with the findings of the authors cited.

The suggestion that the daily protein intake, which is usually at least twice as high with cow's milk formulas as with human milk, might play a rôle was made by Levine, Gordon, and Marples (8) as a result of observations on the amino acid metabolism of infants. They demonstrated the excretion of incompletely oxidized derivatives of phenylalanine and tyrosine in the urine of premature infants whose diet contained a relatively high protein intake (5 grams or more per kilogram per day) and of full-term infants to whom these amino acids were administered when no vitamin C supplements were being given, and they further observed that sufficiently large doses of ascorbic acid diminished or abolished the excretion of the derivatives (8, 9, 10). These results indicate that vitamin C plays an important rôle in the intermediary metabolism of aromatic amino acids, an interpretation consistent with the findings of Seacock and his colleagues (11, 12) that scorbutic guinea pigs also excrete derivatives of tyrosine and phenylalanine, and that the feeding of tyrosine increases the ascorbic acid requirement of these animals. Ley (13), in connection with clinical observations in the newborn, made the suggestion that the individual infant's requirement for this

vitamin may be related to activity and to intermediary metabolism.

As a practical means of investigating the relationship between diet in infancy and vitamin C requirement, two groups of premature infants

were studied, one of which received boiled human milk and the other cow's milk formulas. By means of a modified Kadji test (14) the ascorbic acid stores of both groups following saturating doses of the vitamin were determined.

TABLE I
Ascorbic acid storage in premature infants on different intakes of protein
INFANTS RECEIVING HUMAN MILK

Name	Sex	Birth weight	Protein average	"Saturation dose" of ascorbic acid		Day of test		Days elapsed between saturation and test	Test dose		Plasma ascorbic acid level	
				Total	Days	Age	Weight			Route	Before test dose	4 hours after test dose
		kgm.	grams per kgm. per day	mgm.		days	kgm.		mgm.		mgm. per 100 cc.	
J. H.	F	2.38	2.6	800	4	16	2.60	6	100	Intramuscular	0	1.6
B. U.	F	2.12	2.7	900	5	13	2.41	5	100	Intramuscular	0.3	1.3
H. R.	M	2.34	2.7	900	5	19	2.52	7	100	Intramuscular	0	1.7
A. S.	F	2.13	2.7	900	5	17	2.24	7	100	Intramuscular	0.5	3.5
R. Pl.	F	2.00	2.8	800	4	26	2.44	6	200	Oral		2.6
J. R.	M	2.33	2.7	800	4	16	2.48	7	200	Oral		1.4
R. Pu.	M	1.71	2.7	800	4	15	1.98	7	200	Oral		1.8
C. C.	F	2.28	2.7	800	4	22	2.56	7	200	Oral		1.5
D. C.	F	2.09	2.6	800	4	19	2.33	7	200	Oral		1.9
A. C.	F	1.99	2.7	800	4	19	2.17	7	200	Oral		2.0
P. G.	M	1.98	2.6	800	4	21	2.45	7	200	Oral		1.9
C. B.	F	1.84	2.7	800	4	16	2.13	6			0.8	
F. D.	F	1.75	2.7	900	5	20	2.03	7			0.1	
											Average	1.9

INFANTS RECEIVING COW'S MILK FORMULAS

Name	Sex	Birth weight	Feeding		"Saturation dose" of ascorbic acid		Day of test		Days elapsed between saturation and test	Test dose		Plasma ascorbic acid level	
			Protein	Type of milk*	Total	Days	Age	Weight			Route	Before test dose	4 hours after test dose
		kgm.	grams per day per kgm. body weight		mgm.		days	kgm.		mgm.		mgm. per 100 cc.	
T. M.	M	1.80	5.9	PWM	900	5	18	2.11	7	100	Intramuscular	0	0.5
M. S.	F	2.13	5.0	Olac	900	5	20	2.66	7	100	Intramuscular	0	1.0
C. M.	M	1.79	4.6	EM	900	5	19	2.06	5	100	Intramuscular	0.2	1.4
P. V.	F	1.66	4.7	EM	800	4	19	2.16	6	200	Oral		1.2
P. Mo.	F	2.16	6.1	Alacta	800	4	19	2.68	6	200	Oral		0.6
P. Mc.	F	2.27	4.8	EM	800	4	23	2.69	7	200	Oral		0.3
C. T.	F	1.45	6.1	Olac	800	4	45	2.02	7	200	Oral		1.6
C. L.	F	2.23	6.1	Alacta	800	4	19	2.69	7	200	Oral		0.2
S. M.	M	2.31	4.7	EM	800	4	25	2.53	7	200	Oral		0.4
P. J.	F	1.99	4.9	EM	800	4	15	2.28	7	200	Oral		0.1
T. Z.	M	2.05	4.7	EM	800	4	15	2.34	7	200	Oral		1.5
C. G.	F	1.85	7.0	Alacta	800	4	18	2.21	6			0.2	
F. T.	M	2.35	5.9	Alacta	900	5	19	2.53	8			0.1	
											Average		0.8

* Abbreviations PWM and EM refer to powdered whole milk and evaporated milk, respectively.

PROCEDURE AND METHODS

Subjects and diets

The subjects were 26 premature babies whose birth weights and other significant data are tabulated in Table I. Except for the formulas and ascorbic acid,¹ none of the infants received other food or medication except 5 per cent glucose on the first day or two of life, and a concentrate of vitamins A and D beginning usually about the seventh day. No infants who required transfusions were included. One group of infants received pooled human milk obtained chiefly from mothers of infants on the ward and prepared by boiling for 20 minutes in the formula room at the hospital. Formulas of cow's milk of isocaloric value but of varying composition were given to the second group, some being prepared from evaporated milk, some from Olac,² a mixture having the proportions of whole cow's milk but in which the butter fat is replaced by olive oil, and some from Alacta,² a preparation of cow's milk from which more than half of the fat has been removed. All of the latter formulas contained added sugar and all were boiled. Vitamin C analyses were made on occasional samples and will be discussed later.

Ascorbic acid administration

Observation on each infant was begun when he or she reached an age and state of vigor at which he was able to take a full formula providing about 120 to 125 calories per kilogram of body weight per day. The average age was 10 days. In order to saturate the tissues with ascorbic acid, 800 to 900 mgm. were given in divided doses over a period of 4 to 5 days. The vitamin doses were given by mouth in every instance except in 4 infants in whom it was decided to obtain a check on the fact that these enormous doses actually did "saturate" the body. In these 4 infants the final dose was an intramuscular one of 100 mgm. ascorbic acid which was followed by a test similar to that of Kadji, Light and Kadji (14). The results are recorded in Table II.

During an interval of 5 to 8 days following the last dose of ascorbic acid, the infant was given no additional source of the vitamin. The diet during this interval, as well as during the days when the "saturation" doses of vitamin C were given, is shown for each infant in Table I, the figures for protein intake representing the average daily amount for the period. On the test day, ascorbic acid was given, either 100 mgm. intramuscularly in the gluteal region or 200 mgm. by mouth, between 7:30 and 9:30 a.m. Four hours later a venepuncture was performed to obtain blood for the determination of plasma ascorbic acid. In addition, in all of the infants who were

¹ Prior to the "saturation" doses of ascorbic acid listed in Table I, the following infants had received a number of daily doses of 25 mgm. each: infants H. R., C. C., M. S., P. M., C. T., and S. M. received, respectively, 1, 2, 1, 5, 17, and 7 doses. Infant R. Pl. received 25 mgm. daily on the eighth to eleventh days of life and 200 mgm. daily on the twelfth through the fifteenth days.

² Manufactured by Mead Johnson and Co.

TABLE II

Test for saturation on day after four successive daily doses of 200 mgm. of ascorbic acid

Test dose, 100 mgm. intramuscularly.

Subject	Feeding	Plasma ascorbic acid level		
		18 hours after last oral dose	4 hours after test dose	Increase
		mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.
B. U.	H. M.	0.5	2.8	2.3
F. D.	H. M.	0.4	2.6	2.2
H. R.	H. M.	1.2	3.0	1.8
C. M.	E. M.	0.8	3.5	2.7

to receive the intramuscular test dose, a "fasting" plasma level was obtained just before the injection.³

Chemical methods

The method for the blood plasma ascorbic acid determinations was essentially that of Mindlin and Butler (15), using the Evelyn photocolormeter. The method was modified by the omission of cyanide, as this has been shown to be unnecessary by Friedman, Rubin, and Kees (16). Special care was taken to avoid hemolysis of the blood and to chill the samples soon after drawing, as recommended by Snelling and Jackson (17), and Heinemann and Hald's precaution (18) of checking the pH of the metaphosphoric acid reagents by frequent titration was followed. In many instances it was undesirable or impossible to withdraw from the small veins of premature infants sufficient blood to yield 2 cc. of plasma on centrifugation. After a preliminary study of the micro-method described by Mindlin and Butler (15), it was decided that more accurate results could be obtained by a modification of the macromethod, using smaller amounts of plasma, precipitating them with metaphosphoric acid reagent and water in the proportions used in the macromethod, and diluting the filtrates as necessary with 0.22 N metaphosphoric acid before making the colorimeter readings. In agreement with Heinemann and Hald (18), it was found that readings so obtained on an original sample of 0.8 cc. or more of plasma were entirely satisfactory. In a few instances in which turbid filtrates were obtained, the modification suggested by Bessey (19) was used.

Milk samples were analyzed for ascorbic acid by a modification of Bessey's method (19). No ascorbic acid

³ The reason for the change from a parenteral to an oral test dose was the occurrence in 2 infants of a rather severe local reaction characterized by sterile necrosis and sloughing. This was attributed to the acidity of the solution and the possibility that, due to the small size of the gluteal muscles in premature infants, a portion of the dose may have inadvertently been injected subcutaneously instead of intramuscularly.

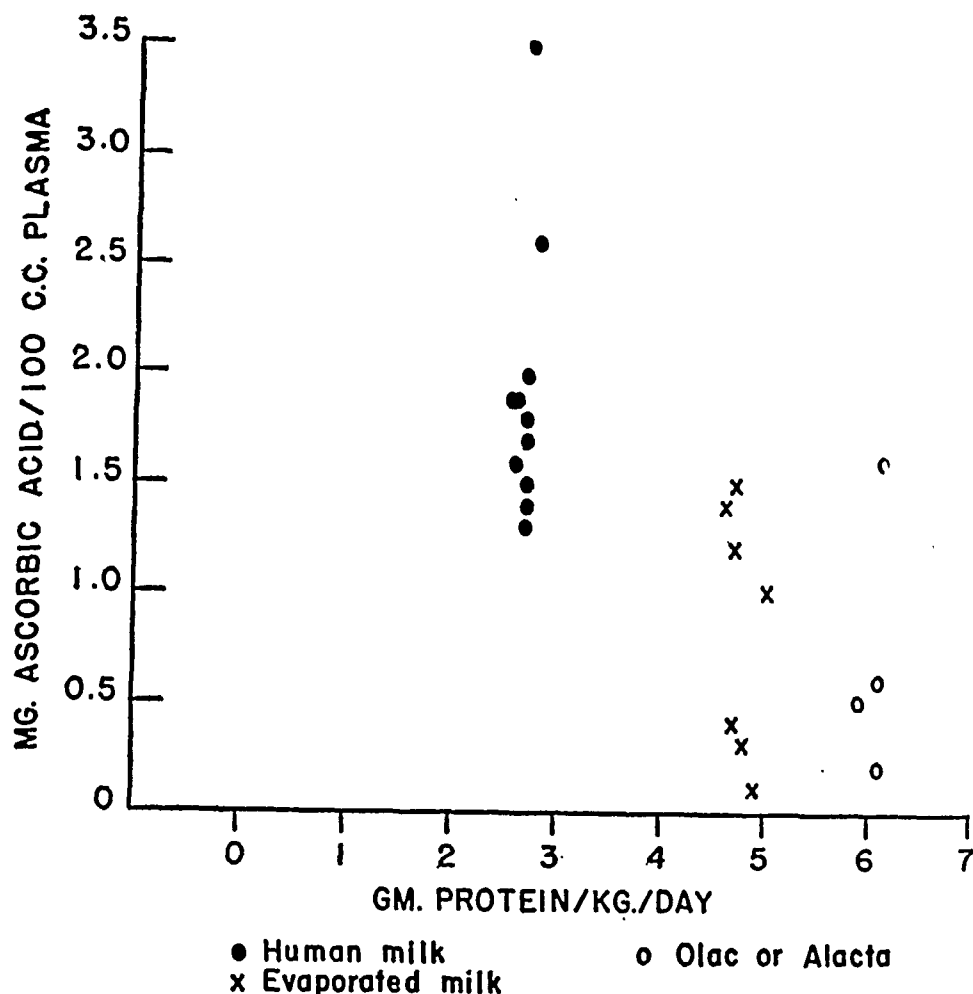


FIG. 1. RELATION BETWEEN PROTEIN INTAKE AND PLASMA ASCORBIC ACID LEVEL, 4 HOURS AFTER TEST DOSE

was found either in boiled human or boiled cow's milk.⁴ A few attempts were made to use the method of Woessner, Elvehjem, and Schuette (20), which proved satisfactory except in a few instances in which a heavy turbidity could not be prevented.⁵

RESULTS

The findings are in accord with the opinion expressed by Mindlin (7), Kadji, Light, and Kadji

⁴ We are indebted to Dr. Walter Golden for some of the milk analyses.

⁵ In two human milk samples, very turbid filtrates were obtained. Prior to correction, the ascorbic acid values corresponding to the galvanometer readings were actually less than zero, but after Bessey's correction (19) was applied apparent reducing activity was present equivalent to 1.6 and 1.3 mgm. of ascorbic acid per 100 cc. If this were actually ascorbic acid, the maximum intake would have been 2.6 mgm. of ascorbic acid per kilogram per day, an amount which might conceivably aid in retention of tissue stores of the vitamin. However, it seems unlikely that ascorbic acid in reduced form could be present after 20 minutes of boiling in contact with air, especially since none was detected in milk specimens in which turbidity did not interfere.

(14) and others, that the response to a test dose is more significant than single plasma ascorbic acid determinations. The latter show much variation even in infants of presumably the same nutritional status. For example, in Table II the "fasting" levels (18 hours after the last large dose of vitamin C) ranged from 0.4 to 1.2 mgm. per cent. Two additional plasma samples obtained from infants C. B. and C. G. under the same conditions contained 0.9 and 0.5 mgm. per cent, respectively. On the day of the final test, after 5 to 8 days without ascorbic acid, "fasting" levels ranged from 0 to 0.8 mgm. per cent (Table I, next to last column). Little significance can be attached to these values except that, as might be expected, the 5- to 8-day interval invariably resulted in a lower "fasting" level.

Plasma values at a suitable interval after a test dose (in this study, a 4-hour interval) are a more reliable index of the true state of the body with respect to vitamin C storage. This is illustrated in Table II. In babies who were fully saturated,

the plasma ascorbic acid after a test dose rose to 2.6 to 3.5 mgm. per cent.

Table I shows the results after a 5- to 7-day "depletion" interval in 22 babies, half of whom received boiled human milk and the others formulas made of cow's milk. It is at once apparent that the former showed a much greater response to the test dose (Table I, last column). The values for the infants receiving human milk ranged from 1.3 to 3.5 mgm. per 100 cc. of plasma, while the others ranged from 0.1 to 1.6 mgm. per cent. The average for the former group was 1.9 and for the latter 0.8 mgm. per 100 cc. If exceptions are made of the 2 infants, R. Pl. and C. T., who happened to receive large amounts of vitamin C prior to the "saturation" dose,¹ the averages become 1.86 and 0.72 mgm. per 100 cc. for the "human milk" group and the "cow's milk" group, respectively. All of the babies fed human milk showed a plasma response to the test dose of at least 1.2 mgm. per 100 cc., while of the babies receiving cow's milk formulas only 3, one of whom was subject C. T. already mentioned, were above this level.

Figure 1 shows graphically the difference between the two groups which have been plotted so as to show the correlation between the response to the test dose of ascorbic acid and the level of protein intake. No such correlation was found with age, sex, birth weight, weight on the day of the test, or with any other variable except the type of feeding.

DISCUSSION

The greater response to the test dose of ascorbic acid in the group of infants receiving human milk than in those receiving cow's milk formulas is interpreted as meaning that the former were able to retain a greater amount of the "saturation" doses of the vitamin in their tissues. Presumably, either something in human milk has a "sparing" effect on the vitamin or something in cow's milk calls for a more rapid use of it, or both factors play a part. The presence of ascorbic acid, as such, which may be abundant in fresh breast milk, seems almost surely eliminated by the prolonged boiling of the human milk used in this study.⁵

The hypothesis is suggested that the vitamin C requirement of infants is raised as a result of a

difference in composition of human milk and of cow's milk formulas. The most striking difference is that in protein content, human milk having about 1.5 grams of protein per 100 cc., and therefore providing, in the amounts given to these premature infants, from 2.6 to 2.8 grams per kilogram per day, while the cow's milk formulas contained amounts providing from 4.6 to 6.1 grams per kilogram per day.

Linking the vitamin C requirement to the protein in the diet is consistent with the aforementioned observation of Sealock, Ziegler, and Driver (12) that guinea pigs given tyrosine in their diets require more ascorbic acid than controls, and with the findings of Levine, Gordon, and Marples (8) that the aromatic amino acids tyrosine and phenylalanine are incompletely metabolized by premature babies in the absence of supplements of ascorbic acid. Whether the vitamin plays a rôle in the oxidative processes of intermediary metabolism, or in the building of body protein, or both, is beyond the scope of this paper.

SUMMARY

Following a standard procedure of saturation with ascorbic acid, and a subsequent period of no ascorbic acid, the response of the blood plasma ascorbic acid level to a test dose of the vitamin was used as a criterion of the degree of saturation of the tissues. Observations were made in premature infants receiving boiled breast milk or artificial formula feedings.

Eleven infants given human milk showed plasma ascorbic acid values ranging from 1.3 to 3.5 mgm. per 100 cc. 4 hours after a test dose of either 100 mgm. of ascorbic acid intramuscularly or 200 mgm. by mouth, the average value being 1.9 mgm. per cent. Eleven infants whose feedings consisted of various cow's milk formulas responded to test doses with values having a range of 0.1 to 1.6 mgm. per cent, only 3 being above 1.2 mgm. per cent; the average was 0.8 mgm. per cent.

The results are interpreted as signifying that premature infants receiving human milk retain a larger part of a "saturation" dose of ascorbic acid in their tissues than do infants given cow's milk. The hypothesis is offered that an increased daily requirement for vitamin C is related to a high level of protein intake. This explanation is in accord

with available evidence that ascorbic acid is concerned in the intermediary metabolism of aromatic amino acids.

BIBLIOGRAPHY

1. Smith, S. L., *Human requirements of vitamin C*. J. A. M. A., 1938, 111, 1753.
2. Selleg, I., and King, C. G., The vitamin C content of human milk and its variation with diet. J. Nutrition, 1936, 11, 599.
3. Braestrup, P. W., The content of reduced ascorbic acid in blood plasma in infants, especially at birth and in the first days of life. J. Nutrition, 1938, 16, 363.
4. Mindlin, R. L., The relation between plasma ascorbic acid concentration and diet in the newborn infant. J. Pediat., 1938, 13, 309.
5. Mindlin, R. L., Variations in the concentration of ascorbic acid in the plasma of the newborn infant. J. Pediat., 1940, 16, 275.
6. Snelling, C. E., The plasma ascorbic acid of infants and children. J. Pediat., 1939, 15, 824.
7. Mindlin, R. L., Concentration of ascorbic acid in the plasma during the treatment of infantile scurvy. J. Pediat., 1940, 17, 621.
8. Levine, S. Z., Gordon, H. H., and Marples, E., A defect in the metabolism of tyrosine and phenylalanine in premature infants. II. Spontaneous occurrence and eradication by vitamin C. J. Clin. Invest., 1941, 20, 209.
9. Levine, S. Z., Marples, E., and Gordon, H. H., A defect in the metabolism of aromatic amino acids in premature infants: the rôle of vitamin C. Science, 1939, 90, 620.
10. Levine, S. Z., Marples, E., and Gordon, H. H., A defect in the metabolism of tyrosine and phenylalanine in premature infants. I. Identification and assay of intermediary products. J. Clin. Invest., 1941, 20, 199.
11. Scalock, R. R., and Silberstein, H. E., The excretion of homogentisic acid and other tyrosine metabolites by the vitamin C-deficient guinea pig. J. Biol. Chem., 1940, 135, 251.
12. Scalock, R. R., Ziegler, B., and Driver, R. L., The relation of vitamin C to the metabolism of the melanin pigment precursors, tyrosine and dihydroxyphenylalanine. J. Biol. Chem., 1939, 128, lxxxix.
13. Ley, L., Die Bedeutung des Vitamin C für das Neugeborene. Klin. Wchnschr., 1937, 16, 1425.
14. Kadji, L., Light, J., and Kadji, C., A test for the determination of the vitamin C storage. J. Pediat., 1939, 15, 197.
15. Mindlin, R. L., and Butler, A. M., The determination of ascorbic acid in plasma; a macromethod and micromethod. J. Biol. Chem., 1937-8, 122, 673.
16. Friedman, G. J., Rubin, S. H., and Kees, W., Effect of addition of KCN to whole blood on indophenol-reducing power of plasma. Proc. Soc. Exper. Biol. and Med., 1938, 38, 358.
17. Snelling, C. E., and Jackson, S. H., Blood studies of vitamin C during pregnancy, birth, and early infancy. J. Pediat., 1939, 14, 447.
18. Heinemann, M., and Hald, P. M., Factors that influence the passage of ascorbic acid from serum to cells in human blood. J. Clin. Invest., 1940, 19, 469.
19. Bessey, O. A., A method for the determination of small quantities of ascorbic acid and dehydroascorbic acid in turbid and colored solutions in the presence of other reducing substances. J. Biol. Chem., 1938, 126, 771.
20. Woessner, W. W., Elvehjem, C. A., and Schuette, H. A., The determination of ascorbic acid in commercial milks. J. Nutrition, 1939, 18, 619.

BLEEDING TIME, LYMPH TIME, AND CLOT RESISTANCE IN MEN¹

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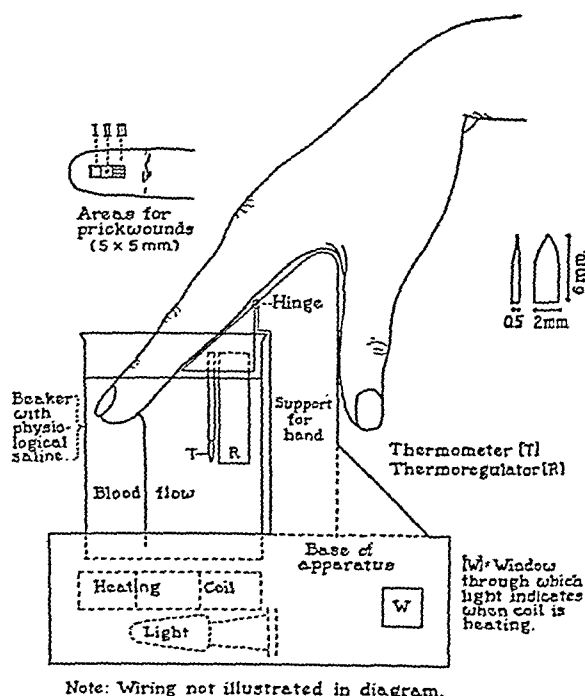
Hemostasis in a skin wound is measured by the bleeding time. This phenomenon was first observed by Duke in 1910 (1). Improvements in the bleeding time technique, as described by Duke (1, 2), have since been made. Roskam (3) washed the blood emerging from the wound with a fine jet of water at an even pressure and temperature in order to avoid disturbing the lips of the wound by blotting. Ivy, Shapiro, and Melnick (4) attempted to eliminate the variable of capillary tonus by applying the cuff of a sphygmomanometer around the arm with a pressure of 40 millimeters of mercury. Tocantins (5) devised an instrument with which he produced a uniform wound on the dorsal surface of the forearm. Dishoeck and Jongkees (6) produced surface skin wounds 4 mm. in diameter on the lobe of the ear, thereby cutting the capillaries without injuring the larger vessels.

In order to control most of the known factors which influence the bleeding time, we felt it necessary to adopt the principle introduced by Döttl and Ripke (7) of bleeding into fluid. Variations in temperature have been shown by König (8) and Roskam (3) to influence the bleeding time in men, and by Döttl and Ripke (7) and the authors (9) to influence the bleeding time in mice. In carrying out these tests the temperature was constantly kept at 37.5° C. Changes of venous pressure, movements of the hand and arm, and pressure on the wound margins in the process of blotting the blood have been eliminated. We were unable to control the size and the number of vessels cut. Nevertheless, we attempted to keep more constant the factors of skin elasticity and the number and size of vessels cut by inflicting deeper puncture wounds than are commonly employed. The objective was

to devise a clinical method in which the experimental conditions have been standardized with reference to the variables which can affect the bleeding time. The application of bleeding into fluid permitted observations of new phenomena which we present.

METHODS

A constant temperature bath, capable of maintaining 200 cc. of isotonic saline at 37.5° C. was constructed. We have called this portable bath with a support for the hand the hemorrhagometer (Figure 1). The terminal phalanx of the third or fourth finger was cleaned with alcohol and immersed for 2 minutes into the bath to attain



Note: Wiring not illustrated in diagram.

FIG. 1. HEMORRHAGOMETER

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² Aided by a grant from the Dazian Foundation for Medical Research.

³ George A. Breon Fellow in Experimental Medicine.

the temperature of the saline bath. Usually prick wounds of the finger tip inflicted with a stylet measure from 2.5 to 3.5 mm. in depth. We found that it was necessary to use a stylet with a strong spring and produce a wound depth of 6 mm. to insure a free flow of blood. A mechanical stylet (Penn U.S.A. Chrome) with blade dimensions 0.5 x 2 x 6 mm. was used (Figure 1). We had

difficulty in maintaining the sharpness of the blade since commercial stylets are constructed of untempered steel. Area I of the finger, as shown in Figure 1, is to be preferred, since injury to the periosteum or bone is least likely. The heated phalanx was removed temporarily while the prick wound was inflicted. During the test the subject sat quietly in an armchair. The position of the hand was kept at a level of 10 to 15 cm. below the base of the subject's heart to eliminate changes in venous pressure. The bleeding time was measured with a stop watch from the moment the wound was inflicted until the flow of blood stopped.

Two types of blood flow were differentiated following the production of the puncture wound: a non-pulsating, and a pulsating flow synchronous with the radial pulse. The pulsating flow may emerge with great force, perpendicular to the long axis of the finger; or it may flow directly down into the container. Not all perpendicular flows had pulsations synchronous with the heart beat. According to the amount of delivery of blood from the wound, the blood flow was further differentiated into various strengths: very strong (ss), strong (s), moderate (m), feeble (f), and very feeble (ff). Usually the flow of blood diminished in strength or volume until hemostasis occurred. In the Tables the listed strength of flow indicates the maximum output of blood which occurred during the test. Occasionally we observed fluctuations, feeble flows being followed by strong ones.

The influence of various temperatures on the bleeding time was investigated, using saline solutions of the following temperatures: 12.5, 25, 37.5, and 50, $\pm 1^\circ$ C.

We observed, in addition to the red or blood flow, a whitish flow which may be observed alone or simultaneously with the red flow. The whitish flow, which we believe is a mixture of lymph, tissue fluid, and plasma was measured from the time the wound was inflicted until the flow stopped. We propose to name the duration of this whitish flow the "lymph time", in differentiation from the bleeding time. It may be noted that for the observation of the "lymph time", strong illumination and a clear physiological saline solution are required. At times this whitish flow may be interspersed with bead-like masses of blood and thus assumes a thread-like appearance.

A latent period was measured from the infliction of the prick wound until the blood flow started. Clot appearance at the site of the wound was observed during and following the bleeding time. The size and appearance of this external clot were studied.

The effect of capillary tonus (4) was investigated by applying a cuff-pressure of 40 mm. Hg around the arm, after which the bleeding time and "lymph time" were determined. In another series of investigations, immediately after the "lymph flow" had stopped, a cuff-pressure of 100 mm. Hg was applied for 3 minutes in order to observe whether a blood flow or "lymph flow" could be provoked.

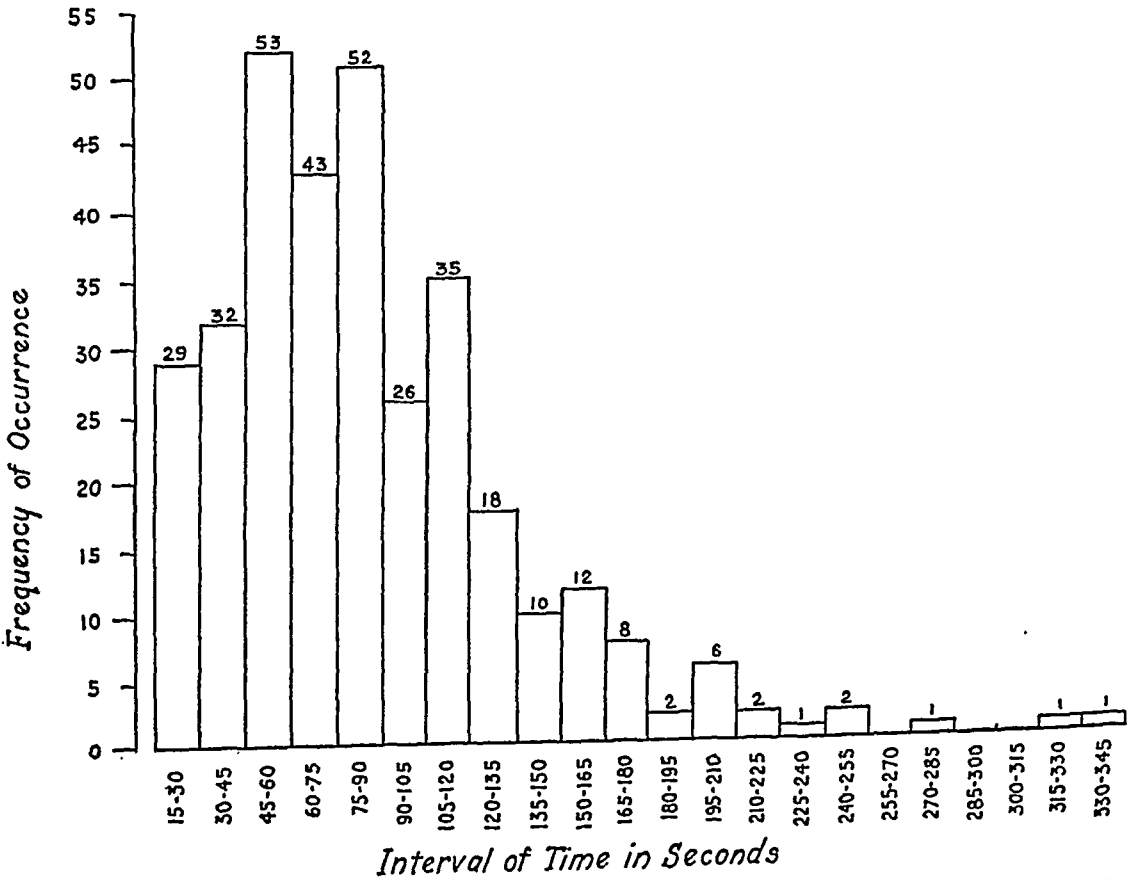


FIG. 2. FREQUENCY DISTRIBUTION OF 334 DETERMINATIONS OF BLEEDING TIME IN 174 SUBJECTS

RESULTS

Bleeding time

Figure 2 illustrates the frequency distribution of 334 single determinations of bleeding time in 174 subjects composed of medical students, the clinic staff and ambulatory clinic patients. The bleeding times ranged from 17 to 340 seconds. Eighty-seven per cent were from 30 to 180 seconds, and 95 per cent fell between 17 and 180 seconds. Five per cent of the values were found to be over 180 seconds. We consider bleeding time values between 3 and 6 minutes as slightly prolonged, and values over 6 minutes as pathological. Usually the latent period is from 0 to 4 seconds. Since most of the latent periods were less than 4 seconds, we included them in the bleeding time values.

TABLE I

Agreement of bleeding times taken within an hour

Agreement within seconds	1-15	15-30	31-45	46-60	61-75	76-90	91-105	121-135	Total
Frequency of occurrence	62	42	19	11	7	2	1	1	145
Occurrence in per cent	42.7	29.0	13.1	7.6	4.8	1.4	0.7	0.7	100

Table I shows how the bleeding time values differ in two or more successive tests done within an hour. In those instances where three or four tests were done, an average was computed and the deviation from the average was expressed in seconds. It is shown that out of 145 comparisons, 104 of the values checked within ± 30 seconds.

In Table II bleeding times are presented which were done on 10 individuals on successive occasions after intervals of days or months. On the same day variations from 67 to 197 seconds occurred in Case N. N. and from 54 to 204 seconds in Case A. L. C. These values show that infrequently wide divergencies may occur; however, tests over a longer interval of time agree closely. In such instances where one value was much less than others, we found it was usually due to the inflection of an inadequate puncture wound.

Table III shows the effect of temperature on the bleeding time. In order to lessen trauma only two tests were done on one finger. It is evident

TABLE II

Repeated bleeding times in ten subjects

Subject	Date 1940-1941	Number of tests	Bleeding time in seconds			
T. P. R.	December 6 December 10	2 2	f 60 s 71	f 62 ss 69		
N. N.	November 22 December 3 December 10	4 1 2	s 197 ff 141 s 135	s 67 f 177	s 144	s 159
E. C.	November 14 December 10 May 23 May 29	2 2 1 1	f 67 m 114 m 76 ss 39	f 68 m 109		
B. L.	November 20 December 6 June 26	2 2 1	f 128 ff 123 m 200	s 154 I ss 246 P		
V. P.	November 4 December 10 July 5	1 2 1	m 35 f 41 s 55	f 60		
E. C. K.	December 12 June 26 August 25	1 2 1	m 325 m 31 ss 45	s 45		
L. C.	December 6 August 22	2 1	ff 47 f 44	m 66		
D. M. C.	December 6 June 5 June 26 August 22	3 1 2 1	m 215 ss 160 ss 330 P ss 131	m 230 ss 170	ss 279	
J. J. L.	September 15 November 14 November 19 November 20 December 10 April 8 August 22	2 2 1 2 2 2 1	48 f 27 m 73 m 110 20 ff 36 s 64	77 f 69 ss 90 47 f 32		
A. L. C.	September 15 November 14 November 17 November 19 December 6 March 26 August 18 August 22	2 2 1 1 3 2 1 2	34 s 66 ss 84 m 63 m 54 ss 249 P ss 223 P s 62	35 s 80 ff 62 m 70 ss 53	m 204 I	

ss = very strong, s = strong, m = moderate, f = feeble, ff = very feeble; I = intermittent fluctuation in strength of flow; P = pulsating flow.

from this table that temperatures lower than 36.5° C. prolong the bleeding time. Tests which were done at 50° C. agreed closely with the values which

TABLE III

The effect of temperature upon the bleeding time

Subject	12.5° C.	25° C.	37.5° C.	50° C.
	seconds	seconds	seconds	seconds
J. G. S.	390	116	20	146 P
L. C. K.	275	151	21	15
G. A. B.	350	198	22	34
R. S.	163	72	23, 68 P	55
D. A. B.	356 P	126	30	46
D. L.	185	154	40	42
L. C.	259	246 P	44	52
M. H. L.	126	127 P	45	31
H. B.	532 P	139	51	43
A. L. C.	521	216 P	58	52
J. J. L.	203	103	64	107
M. S.	398 P	250	82	33
D. M. C.	522 P	462 P	131	139 P

P = pulsating flow.

were obtained at 37.5° C. We have found a relative increase in pulsating flows at lower temperatures. Pulsating flows augment the effect of lower temperatures by prolonging the bleeding time. Variations in the volume output of blood from the wound occurred more frequently at lower temperatures than at 37.5° C. The latent periods were not significantly affected by changes in temperature.

Two to four repeated tests performed at different temperatures were obtained on 7 subjects. Values which were obtained at 37.5° C. agreed closely, whereas this was less likely to be the case when bleeding times were done at 25° C.

Of 345 bleeding times on 183 subjects without cuff pressure, eleven pulsating flows were seen which varied from 56 to 330 seconds, with an average of 183 seconds. With a cuff-pressure of 40 mm. Hg in 53 persons, pulsating flows occurred 23 times, with variations from 46 to 330 seconds and with an average of 165 seconds. These results in normal individuals show that pul-

sating flows are not appreciably longer than the usual or non-pulsating flows.

Table IV exhibits a comparison of the bleeding time done in the same subject with and without a cuff-pressure of 40 mm. Hg. The same finger was used in two successive tests. The results in 25 of 53 subjects who were studied for this comparison are shown. In 3 cases bleeding time was decreased from 114 to 44 seconds following cuff-pressure. Values which checked within 30 seconds were observed in 10 cases, while in 11 cases there was an increase of 43 to 150 seconds. In the remaining 28 cases, not shown in this table, this relationship was essentially the same. The only prolonged bleeding time we observed (Case 25) occurred in a case of thrombopenia (platelets 45,000, Lec and White coagulation time 9 minutes, Duke bleeding time 2 minutes). Latent periods were usually shortened by cuff-pressures of 40 mm. Hg. However, in 7 of 53 instances latent periods were found between 6 and 24 seconds.

New phenomena and their relation to bleeding time

"Lymph time" tests were made on 73 persons. The values ranged from 0.5 to 50 minutes. Figure 3 shows the frequency distribution of "lymph time" values which ranged from 0.5 to 19 minutes in 68 persons. In 17 individuals the effect of 40 mm. Hg cuff-pressure on the "lymph time" was studied. This cuff-pressure was found to increase the "lymph time" slightly in the majority of instances.

The phenomenon of clot appearance over the mouth of the wound was observed. Bleeding time, "lymph time", and clot appearance were compared in 53 subjects. Twenty-five of those are shown in Table V. No apparent correlation exists between the bleeding time and the "lymph time". Invariably the "lymph time" is of longer duration than the bleeding time. In 6 subjects no external clot was seen after the bleeding time and "lymph time" measurements were completed. In 8 other cases external clots which had formed towards the end of the bleeding time test did not change in size during the period of "lymph flow". In 8 subjects in whom no clot was visible at the termination of the bleeding time, clot appearance was observed towards the end of the "lymph flow".

TABLE IV

The effect of 40 mm. Hg of cuff-pressure on the bleeding time in twenty-five subjects

Number	Without cuff-pressure		With cuff-pressure		Difference in seconds
	Latent period	Bleeding time	Latent period	Bleeding time	
	seconds	seconds	seconds	seconds	
1	3	m I 270	.2	m 156	-114
2	2	m 99	24	m 54	-45
3	1	s 340	2	s 296	-44
4	3	s 60	9.5	m 31	-29
5	3	ss 55	2	s 31	-24
6	3	m 77	2	s 56	-21
7	3	m 80	2	ss 72	-8
8	1	ss P 330	1	ss P 330	0
9	3	s 54	4	s 63	+9
10	2	m 106	4	m 119	+13
11	0	ss 56	3	s 72	+16
12	3	s 52	3	s 78	+26
13	3	s 82	3	s 111	+29
14	4	m 37	2	s 80	+43
15	4	m 27	3	ss 72	+45
16	3	ss 107	2	ss 153	+46
17	4	s 37	1	s 90	+53
18	7	m 58	6	ss 112	+54
19	2	ss P 131	2	s P 196	+65
20	2	s 29	2	m 96	+67
21	2	s P 56	1	ss P 158	+102
22	2.5	s 45	1	ss 165	+120
23	4	m 76	1	s 198	+122
24	3	ss 55	16	s 185	+130
25	3	ss P 550	3	ss P 700	+150

ss = very strong, s = strong, m = moderate; I = intermittent fluctuation in strength of flow; P = pulsating flow.

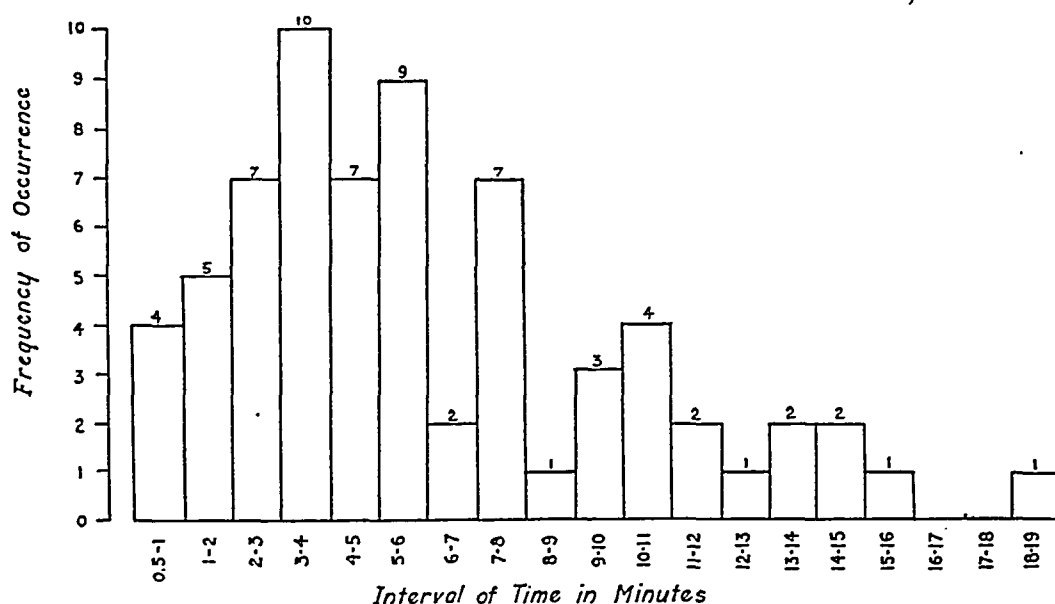


FIG. 3. FREQUENCY DISTRIBUTION OF 68 DETERMINATIONS OF LYMPH TIME IN 68 SUBJECTS
Five subjects not shown in this figure had values which varied from 25 to 50 minutes.

In 3 cases the external clot which was observed at the end of bleeding time enlarged during the flow of "lymph". Table V suggests that clot appearance does not occur with shorter bleeding and shorter "lymph flows".

Experiments with 100 mm. of mercury cuff-pressure

An attempt was made to renew the flow of blood or "lymph" following intervals of 0.5 to 50 minutes after bleeding had stopped. The effects of a cuff-pressure of 100 mm. Hg for 3 minutes were studied on 55 subjects in order to determine whether the clot which had formed inside of the wound would be able to resist such an increase in venous pressure.

In 3 cases the clot was unable to resist this pressure, and a flow of blood started again. These red flows began from 49 to 234 seconds after the blood flow had stopped and lasted from 90 to 240 seconds. In each instance, these blood flows stopped while the pressure was being maintained. In this group of persons it was not possible to make a wound resume bleeding with this pressure 4 minutes after blood flow had stopped.

"Lymph flows" were renewed in 45 instances. In 39 cases the flow of "lymph" continued over the 3-minute period, and in 6 instances "lymph

flow" stopped before the 3 minutes were over. In 7 other cases no "lymph flow" was initiated by the cuff-pressure.

TABLE V
Comparison of bleeding time, lymph time and clot appearance

Case	Bleeding time	Clot after flow of blood	Lymph time	Clot after flow of lymph
	<i>seconds</i>		<i>seconds</i>	
1	ff 18	None	32	None
2	f 25	None	465	Present
3	m 25	None	700	Present
4	m 26	None	118	None
5	s 42	None	635	Present
6	m 45	None	184	None
7	m 47	None	330	None
8	m 52	None	439	None
9	s 54	None	3000	Present
10	ss 56	Present	1620	Enlarged
11	s 62	None	274	Present
12	f 79	Present	184	Not enlarged
13	s 79	None	495	Present
14	f 81	Present	290	Enlarged
15	s 88	None	720	Present
16	ss 108	Present	190	Not enlarged
17	ss 115	None	360	Present
18	ss 130	Present	910	Not enlarged
19	s 157	Present	212	Not enlarged
20	s 158	Present	615	Not enlarged
21	m 160 P	None	270	None
22	m 200	None	320	Enlarged
23	m 243	Present	475	Not enlarged
24	m 285	Present	870	Not enlarged
25	ss 330	Present	480	Not enlarged

ss = very strong, s = strong, m = moderate, f = feeble, ff = very feeble; P = pulsating flow.

The effect of cuff-pressure on the clot appearance was also studied. No change was seen in the external clots which had appeared in 15 instances at the end of bleeding or during the "lymph flow". In 15 other cases, the external clot was enlarged following application of cuff-pressure. In 7 persons no clot appeared after pressure.

During the flow of "lymph" we occasionally observed the whitish flow interspersed with bead-like red masses. These masses may be due either to the escape of blood from opened vessels at intervals or to disintegration of the clot. This flow was produced following application of cuff-pressure in 12 of 55 instances, and it lasted from 15 to 120 seconds. In eleven cases this flow occurred from 2 to 9 minutes following the cessation of the blood flow.

DISCUSSION

The normal bleeding time

The normal Duke bleeding time is between 30 and 180 seconds according to Ivy, Shapiro and Melnick (4) and between 60 to 180 seconds according to Tocantins (5). Ninety-five per cent of our values were between 17 and 180 seconds. One wonders if bleeding times between 3 and 6 minutes, which we designate as slightly prolonged, are pathologic. We cannot answer this question at the present time. Values over 6 minutes, which we regard as pathologic, were found in but one case of thrombopenia. As a rule, the majority of the bleeding times in one individual agree within ± 30 seconds. In the case of E. C. K. (Table II), where $6\frac{1}{2}$ months elapsed between tests, it is possible that the hemostatic function of the skin may have undergone a change during this time. Tests performed at 37.5° C. have shorter bleeding times than at 25° C. and agree more closely when repeated. These findings are, on the whole, similar to data obtained by Dale and Laidlaw (10) in studies on the influence of temperature upon the blood coagulation time. In contrast to the results of Roskam (3), we found that the bleeding time is practically the same at 37.5° C. as at 50° C. The increased incidence of pulsating flow following application of cuff-pressure may be due

to the transmission of the arteriolar pulsation into the capillaries following venous obstruction.

Our method can be used to study hemostatic substances *in vivo*, or locally by adding them to the isotonic saline bath. Furthermore, studies on the fragility of the erythrocytes can be undertaken directly by changing the tonicity of the saline bath.

It is known that following the production of a puncture wound the capillaries contract initially and that dilatation occurs (11). The duration of this initial contraction may be obtained by measuring the latent period. Our findings indicate that the initial capillary contraction may not be eliminated by 40 mm. Hg cuff-pressure. Probably the pain stimulus produced by the infliction of the puncture wound is responsible for these long latent periods.

The lymph time

In the production of the puncture wound not only are the blood vessels injured, but the tissue spaces, cells, and the lymph vessels are also opened. Since we were unable to differentiate tissue fluid from lymph, we assume that these fluids are mixed following injury. It may be noted that the rose coloration of the whitish flow may be due to the admixture of red blood cells with the tissue fluid. We do not believe that this whitish flow is serum from the clot which has formed in the wound for the following reasons: First, the whitish flow can be observed simultaneously with the red flow; second, we have frequently found that an inadequate puncture wound will produce a whitish flow without any blood. The reason why the "lymph" continues to flow after the bleeding stops is probably due to the hypocoagulability of the lymph. It is well established that lymph coagulates (12).

The majority of lymph times are below 16 minutes. The significance of the lymph time cannot be evaluated at present. We suggest that it may be a measure of wound seepage which might affect wound healing. The slightly increased "lymph time" on application of 40 mm. Hg cuff-pressure may be due to the increased lymph flow resulting from the increased venous pressure.

The blood clot

Agglutination of platelets and blood coagulation are two separable processes in mammals (13). We believe that both the agglutination of platelets and the conversion of fibrinogen to fibrin are responsible for the firmness of the clot and its ability to plug the wound. Usually the clot which forms inside of the wound is sufficient to stop the blood flow, although it may not prevent the seepage of "lymph". In the majority of cases, the clot which has formed in the wound extends beyond the wound margins and thus becomes what we have called "the external clot" or "clot appearance." During the flow of "lymph", the external clot may enlarge. A correlation between clot appearance and duration of bleeding time or "lymph time" is suggested in Table V. However, only future investigation can clarify these points.

Tocantins (5) described a method in which he applied negative pressures with a suction cup. He found that a cut in the skin can be made to resume bleeding and suggested that this method might be of use to indicate a bleeding tendency during or after operation. In order to avoid local disturbance of the clot and the wound, we tested the clot resistance against a venous cuff-pressure of 100 mm. Hg for 3 minutes.

Increasing the cuff-pressure to 100 mm. Hg often increased the size of the external clot. This may be due to the increased pressure in the capillary bed and engorgement of the tissue spaces. It is very difficult to dislodge the clot and provoke the flow of blood again once bleeding has stopped in normal individuals. Complete dislodgment of the clot, which was indicated by a renewal of the red flow, occurred in but 3 cases. In no instance was it possible to dislodge the clot 4 minutes, or longer, after the blood flow had stopped. In contrast to this, clot resistance studies in 2 cases of hemophilia differed markedly. In both hemophiliacs whose bleeding times were normal it was possible to renew the flow of blood by applying 100 mm. Hg pressure 75 minutes after bleeding had stopped. The bleeding stopped within 30 seconds, however, when the cuff-pressure was reduced to 40 mm. of mercury. Clot resistance expresses both the solidity of the clot and its ability to adhere to the skin wound. Clot resistance is therefore a measure for hemostasis.

Magnus (14) contended that the all-important factor in the control of capillary bleeding is the ability of the vessel wall to contract. Macfarlane (15), however, while emphasizing that capillary contraction plays the primary rôle in the cessation of bleeding, admits that the solidity of the clot is an important factor. It cannot be denied that the capillaries are involved in the cessation of bleeding from a wound; however, we feel that definite proof is lacking that capillary contraction plays the primary rôle in hemostasis. In this connection it may be of interest to report a case of postoperative shock during which bleeding times were 17 and 30 seconds. We have shown in a previous communication (9) that excessive doses of heparin can produce bleeding tendencies in most mice, and bleeding times longer than 30 minutes in some. Several mice which had a normal bleeding time could still bleed to death from their prick wound if they dislodged the clot which covered the wound. These findings and the results in the two hemophiliacs support the hypothesis that the firmness with which the clot is attached to the wound plays a vital rôle in hemostasis.

SUMMARY

A new method for the determination of bleeding time is described in which the principle of bleeding into fluid is employed. Various factors known to influence the bleeding time were controlled. Blood flows were differentiated with respect both to the volume output of blood from the wound and pulsation of the flow. The range of normal bleeding time for this method was established. The effects of temperature and capillary tonus on the bleeding time were studied.

The phenomena of "lymph time", latent period, a beaded flow of blood, clot appearance, and clot resistance are described. In normal individuals, the clot, when formed, could not be dislodged by applying 100 mm. of mercury cuff-pressure 4 minutes after bleeding had stopped. In 2 hemophiliacs, however, it was possible to dislodge the clot and renew the blood flow 75 minutes after the cessation of bleeding. The mechanism of hemostasis is discussed.

BIBLIOGRAPHY

1. Duke, W. W., The relation of blood platelets to hemorrhagic disease. Description of a method for determining the bleeding time and coagulation time and report of three cases of hemorrhagic disease relieved by transfusion. *J. A. M. A.*, 1910, 55, 1185.
2. Duke, W. W., The pathogenesis of purpura hemorrhagica with especial reference to the part played by blood-platelets. *Arch. Int. Med.*, 1912, 10, 445.
3. Roskam, J., Température et temps de saignement. *Compt. rend. Soc. de biol.*, 1933, 112, 1245.
4. Ivy, A. C., Shapiro, P. F., and Melnick, P., The bleeding tendency in jaundice. *Surg. Gynec. and Obst.*, 1935, 60, 781.
5. Tocantins, L. M., The bleeding time. *Am J. Clin. Path.*, 1936, 6, 160.
6. Dishoeck, H. A. E. v., and Jongkees, L. B. W., Eine neue Methode zur Bestimmung der Blutungszeit. *Klin. Wchnschr.*, 1940, 19, 1216.
7. Döttl, K., and Ripke, O., in *Medicine In Its Chemical Aspects*. Vol. III. Bayer, Leverkusen, Germany, 1938, p. 252.
8. König, L., Versuche über Blutstillung. *Klin. Wchnschr.*, 1922, 1, 2376.
9. Copley, A. L., and Lalich, J. J., The experimental production of a hemophilia-like condition in heparinized mice. *Am. J. Physiol.*, 1942, 135, 547.
10. Dale, H. H., and Laidlaw, P. P., A simple coagulometer. *J. Path. and Bact.*, 1911-1912, 16, 351.
11. Magnus, G., Der Beginn der Entzündung im Bilde direkter Capillarbeobachtung. *Arch. f. klin. Chir.*, 1922, 120, 96.
12. Drinker, C. K., and Field, M. E., Lymphatics, Lymph and Tissue Fluid. Williams and Wilkins. Baltimore, 1933.
13. Best, C. H., Heparin and thrombosis. *The Harvey Lectures*, 1940-41, Series 36, p. 66.
14. Magnus, G., Experimentelle Untersuchungen über den segmentären Gefäßkrampf und den Blutungsstillstand. *Arch. f. klin. Chir.*, 1924, 130, 237.
15. Macfarlane, G. R., Critical review: The mechanism of hemostasis. *Quart. J. Med. (New Series)*, 1941, 10, 1.

THE SIGNIFICANCE OF THE D:N RATIO AND ITS BEARING ON THE MECHANISM OF DIABETES MELLITUS

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The D:N ratio plays a very important part in establishing our present day tenets of intermediary metabolism and it does so particularly in that part of metabolism dealing with the theory of diabetes. At the present time there exists no satisfactory basic theory accounting for the disturbance of metabolism in this disease. One has but to note the extreme divergence of viewpoint of accepted authorities in this field to be aware of this. On the one hand, there are those (1) who state that the diabetic organism can burn glucose as readily as the normal and that the trouble results entirely from the over-production of sugar from non-carbohydrate sources. Opposed to these we have an equally authoritative group (2) who claim that diabetes results simply from the inability of the organism to burn glucose. Much of the divergence of opinion is due to differences in the interpretation of the D:N ratio and of the D:Protein ratio.¹

The general belief that but 58 per cent of protein can be converted to glucose in the body rests on the observation that the D:N ratio of the fasting phlorhizinized dog is 3.65² (3). The claim that the completely diabetic animal cannot utilize glucose rests on the fact that the D:N ratio in this condition approaches this value of 3.65. It is apparent, then, that any error in this basic value should be corrected. The fasting phlorhizinized dog excretes 3.65 grams glucose for each gram of N in the urine and, since one gram of N would come from 6.25 grams protein, it has been concluded that 3.65 grams of glucose is the maximal amount that can be converted from this amount of protein. However, this concept requires that all the glucose so formed in the phlorhizinized animal is excreted and that none is burned by the tissues. Workers responsible for

this view have completely ignored this possibility (4) but it remains nonetheless a necessary postulate of their contention.

On the contrary, there is much to support the view that the tissues of these animals do use a certain amount of glucose. Deuel *et al.* (5) and Wierzechowski (6) have examined this question by administering sugar to fasting phlorhizinized dogs. After the administration of the sugar they obtained a rise in the respiratory quotient which they interpreted as evidence of increased oxidation of carbohydrate. Since the tissues of this preparation are capable of oxidizing glucose given by mouth, it is quite possible that they can oxidize glucose that has its origin from body protein. The fate of glucose arising from protein fed to these animals would give a clue to this. Lusk states (3) that, if meat be fed to the phlorhizinized fasting dog, the urine sugar and nitrogen increase but that the D:N ratio is unchanged. Examination of the protocols of the original reports from Lusk's laboratory (4) shows that usually small amounts of protein were fed so that the nitrogen excretion was increased very little. The effects on excretion of the administered protein, then, would be largely masked by the large basic excretion resulting from the body protein breakdown. Despite this, in several of their experiments the dogs showed a drop in the D:N ratio when protein was fed. Janney and Blatherwick (7) also fed amounts of protein that were small when compared to the basic body protein catabolism. The animals showed slight drops in the D:N ratio as a result of the protein feeding. More recently, Gray *et al.* (8) found some drop in D:N ratio with feeding protein and here again the increase in nitrogen metabolized for the fed period over the fasting period was very small. It is obvious that, if one wished to determine the effect of fed protein on the D:N ratio of the

¹ D:Protein ratio = D:N \times 6.25.

² Or a D:Protein ratio of 3.65/6.25 = 0.58.

phlorhizinized dog, he should feed enough of it at least to double the fasting nitrogen excretion. Since in past experiments this has usually not been the case, such an investigation was undertaken and is reported here.

EXPERIMENTAL

The experiments were carried out on three dogs. Two of the animals were subjected to a control period of 4 or 5 days fasting with the daily injection of 1 gram of phlorhizin suspended in olive oil. The feeding period was then instituted during which time the phlorhizin was continued and the animal was fed amounts of meat or

TABLE I

The effect of protein feeding on sugar excretion of phlorhizinized dog

(1 gram phlorhizin from July 13 on; no food July 10 to 13)

Date	Food	Urine sugar	Urine nitrogen	Urinary D : N ratio	Body weight
	grams	grams	grams		lbs.
July 14	0	18.9	5.7	3.32	
15	0	30.0	8.7	3.45	
16	0	22.6	6.65	3.4	25
17	1300 meat	71.5	28.8	2.48	24.5
18	1300 meat		31.6		
19	1320 meat	84.0	34.0	2.47	27.5
20	1350 meat	94.5	36.8	2.56	
21	900 meat	95.0	33.8	2.70	28.5
22	0	51.2	12.0	4.27	
23	0	30.7	7.7	3.98	
24	0	28.9	7.35	3.93	23.75
25	0	24.1	6.4	3.77	22.75

TABLE II

The effect of protein feeding on sugar excretion of phlorhizinized dog

(1 gram phlorhizin daily from June 20 on; 450 grams meat daily June 21 to 25)

Date	Food	Urine sugar	Urine nitrogen	Urinary D : N ratio	Body weight
	grams	grams	grams		lbs.
June 26	900 meat	60.5	23.1	2.61	28.75
27	860 meat	48.5	15.5	3.13	28.5
28	920 meat	91.5	30.9	2.96	28.75
29	920 meat	81.3	26.9	3.02	28.5
30	0	38.1	8.8	4.33	27.5
July 1	0	35.0	9.85	3.56	27.0
2	0	22.0	6.8	3.24	25.5
3	840 meat	70.5	27.3	2.59	27.0
4	840 meat	73.0	26.7	2.73	26.75
5	920 meat	78.0	26.6	2.93	26.75
6	450 meat	70.5	22.4	3.15	27.25
7	0	34.0	7.2	4.70	
8	0	31.5	9.0	3.5	

TABLE III

The effect of protein feeding on sugar excretion of phlorhizinized dog

(1 gram phlorhizin daily from May 15 on; no food May 15 to 17)

Date	Food	Urine sugar	Urine nitrogen	Urinary D : N ratio	Body weight
		grams	grams		lbs.
May 18	0	23.2	6.46	3.60	
19	0	20.0	6.08	3.28	
20	*Amino acids 550 cc.	30.0	11.9	2.52	
21	*Amino acids 550 cc.	15.95	10.5	1.52	19.5
22	Meat 500 grams	30.5	18.5	1.64	
23	Meat 500 grams	43.4	33.0	1.31	20.0

* A solution of amino acids prepared by hydrolysis of casein containing 1 per cent nitrogen. This product was supplied by Frederick Stearn and Company Laboratory.

amino acids sufficient to give a definite increase in urinary nitrogen. In the other animal the fasting control period was interposed between two feeding periods. The urine sugars for Dogs 1 and 2 were determined by Benedict's method and for Dog 3 by that of Shaffer and Hartmann. Nitrogens were determined by the Kjeldahl method. The meat was usually fed in two portions, at 9 a.m. and at 5 p.m. The results are given in Tables I to III.

DISCUSSION

It is apparent that there is always a drop in the D:N ratio when protein is fed. The average for the fasting periods is 3.50 (not including the first 2 days after food is stopped) and for the feeding periods, 2.40. This brings up the question: if the animal is making 3.5 grams of sugar for each gram body protein nitrogen metabolized when fasting, why does he produce but 2.4 grams sugar when 1 gram of food protein nitrogen is metabolized? The most probable explanation would appear to be that he makes as much sugar from food protein as from body protein but that a certain portion of the glucose that is manufactured by the liver is utilized by the other tissues. Certainly the reactions of the dogs themselves support this view. After 4 or 5 days of fasting and phlorhizin the dogs are usually weak and listless. The eating of the protein results in marked improvement in strength and activity, which is similar to the effect of giving glucose to such an animal and indicates an increased utilization of glucose. Bollman, Mann and Magath (9) have shown that the liver is the site of transformation of amino acids to glucose. This obtains for

amino acids either derived from food or body protein. The liver adds this new formed glucose to the general blood stream. From the blood stream the extra hepatic tissues receive glucose which may have had its origin in food carbohydrate, in body glycogen, or in body or food protein that has been changed in the liver. These tissues could in no way discriminate between glucose molecules of these different origins and, since they utilize glucose that has come from food sources, there is every likelihood that they utilize glucose which has arisen from body protein. And yet such a discrimination by the tissues would have to be postulated if we accept the statement that only 3.7 grams of glucose can come from the protein which gives 1 gram of urinary nitrogen.

The idea that the tissues of the phlorhizinized animal use no glucose appears conjectural and is without adequate experimental support. It is true that the amounts of glucose used are relatively small (10) but this low rate is probably the result of fasting rather than any specific effect of phlorhizin. Bergman and Drury (11) have shown that a fast of 3 to 4 days diminishes markedly the utilization of glucose by the extra hepatic tissues. The phenomenon of "starvation diabetes" first described by Hofmeister (12) could also play a rôle here. It is well known today that, if sugar is given to an animal that has been fasting for some time, very little of it is utilized (13). Furthermore, these animals always show a low blood sugar level and it is possible that the hypoglycemia itself may lower the rate of glucose utilization. There is, then, adequate experimental explanations for the low glucose utilization rate of the tissues of the fasting phlorhizinized dog without having recourse to the conjecture that phlorhizin itself prevents glucose oxidation completely. When the phlorhizinized animal is fed, as our results show, his tissues begin again to use more glucose.

EXPERIMENTS ON DEPANCREATIZED DOGS

The depancreatized animal has also been used for the purpose of determining the protein-to-sugar ratio. In the past it was assumed that the tissues of this preparation used no glucose. In the light of recent work this assumption can no longer be maintained. When the liver of the dia-

betic animal is removed, the blood sugar falls and glucose must be administered to keep the animal from becoming hypoglycemic. The brain of the diabetic animal utilizes glucose almost exclusively and its cells die if they do not have this substance to burn (14). The resting muscle of the diabetic uses glucose (15). We have here evidence that at least two tissues of the body use glucose at all times. It is obvious that any attempt to evaluate the D:N ratio of the depancreatized animal must include an estimation of the glucose utilized by the tissues in addition to that which is excreted.

To this end the following experiments were carried out:

After pancreatectomy dogs were kept on food and insulin for about one month in order to have good healing and recovery. They were then carried for the experimental period which consisted essentially of determining the amount of glucose excreted as a result of protein feeding. Control periods were run for the purpose of correcting for the sugar utilization of the tissues. This was done by determining the amount of sugar that had to be fed to give the same amount of glycosuria. For the experimental periods of our first animals we withheld insulin completely, but this resulted in an unsatisfactory condition of the dogs when fed meat. They developed marked ketosis, vomited and lost desire for food. Two of these animals died. We found that a small daily dose of protamine insulin kept the dogs in excellent condition and with good appetites, without lowering the glycosuria to any great extent. All urine sugars were done by the method of Shaffer and Hartmann. The first dog studied was given 700 grams of lean meat daily for 8 days, then 100 grams sugar daily for 3 days and finally 850 grams meat daily for 8 days. Five units of protamine insulin

TABLE IV

Comparison of effect of sugar and protein feeding on sugar excretion of depancreatized dog

Food per day	Number of days	Insulin units per day	Urine sugar	Urine nitrogen	Urinary D:N ratio
<i>grams</i>			<i>grams per day</i>	<i>grams per day</i>	
700 meat..	8	5	37.8	17.2	2.2
100 sugar..	3	5	49.0	2.3	
850 meat..	8	5	66.0	22.8	2.9

per day were given throughout. The averages for the three periods are given in Table IV. The results of the first day of each period are not included in the averages because of the well-known delay in attaining a steady state when one changes from a high to a low protein intake or vice versa (16). In the second period the tissues must have used 51 grams of sugar daily plus a small amount which might have resulted from the catabolism of the protein represented by 2.3 grams of urinary nitrogen. In the protein feeding periods the tissues must have utilized about the same daily amount of glucose (probably a little less in the first period and a little more in the third) since the average blood sugar levels must have been about the same as during the second period in order to give the same average glycosuria. The averages for the first and third periods are: urine sugar 51.6 grams and urine nitrogen 20.0 grams. We can assume, then, that the protein that gave 20 grams urinary nitrogen gave rise to as much glucose as did the 100 grams fed sugar plus the protein that gave 2.3 grams urinary nitrogen in the second period. In order to get the ratio between sugar and nitrogen we may say: 20 grams protein N is equivalent to 100 grams sugar plus 2.3 grams protein N.

Subtracting 2.3 grams protein N from both sides, we obtain 17.7 grams protein N which is equivalent to 100 grams sugar. This correction for the nitrogen excreted during the sugar feeding period must be made regardless of the view one may have as to what it represents in actual metabolism. If it represents body protein catabolized to form sugar, then there was that much additional sugar produced in that period. If it results from metabolic processes not giving rise to sugar, then we must assume that the same processes operated during the first and third periods, giving rise to the same amount of urinary nitrogen which did not contribute to the glucose produced. This portion should be subtracted from that connected with the sugar formation. We believe that both factors probably operate to produce this nitrogen in the sugar feeding period. These calculations give us the conclusion that 17.7 grams protein nitrogen is equivalent to 100 grams sugar. This is a D:N ratio of 5.65 or a D:Protein ratio of 0.90.

A similar experiment was carried out on another depancreatized dog and the results are given in Table V. The calculations for this experiment are not as simple as in the first case since even on the higher meat intake he did not excrete as much

TABLE V
Comparison of effect of sugar and protein feeding on sugar excretion of depancreatized dog

Food per day	Number of days	Insulin units per day	Urine sugar	Urine nitrogen	Urinary D:N ratio
grams			grams per day	grams per day	
50 sugar..	5	3	46.3	1.76	
200 meat..	4	3	23.8	5.59	4.25
400 meat..	2	3	38.7	10.8	3.58

glucose as during the sugar feeding period. However, on the sugar regime the tissues utilized 50 — 46.3 or 3.7 grams sugar plus what was produced in the processes, giving rise to 1.76 grams nitrogen. On the high meat regime (400 grams meat) we can assume that the tissues utilized close to this amount: let us say 3 grams plus the same amount connected with the 1.76 grams nitrogen. If, then, as before, we subtract the 1.76 grams N of the control period from 10.8 grams N of the protein period, we obtain 9.04 grams N which is equivalent to (38.7 plus 3) grams sugar and which gives us an actual D:N ratio of 4.6. The results for the lower meat intake period (200 grams meat) may be similarly treated except that here we must assume that even less sugar was used by the tissues. However, even if we do not add anything to the urine sugar for this correction, we obtain (5.59 — 1.76) grams nitrogen which is equivalent to 23.8 grams sugar or a D:N ratio of 6.2 or a D:Protein ratio close to 1.

Two other experiments were carried out in this way with diabetic dogs. In these, fat-free meat was fed in place of ordinary lean meat. This was done in order to avoid any possible effect that even the small amount of fat in lean meat may have had. There is little likelihood of this since fat fed to diabetic dogs does not increase their sugar excretion. The meat was treated with alcohol, dried and powdered, then extracted with petroleum ether and finally with diethyl oxide. The results are given in Table VI. The levels of glycosuria are lower for the protein regime than

TABLE VI

Comparison of effect of sugar and protein feeding on sugar excretion of depancreatized dog

(Dog 6)

Food per day	Num-ber of days	Insulin units per day	Urine sugar	Urine nitrogen	Urinary D:N ratio
<i>grams</i>			<i>grams per day</i>	<i>grams per day</i>	
55 sugar.....	6	2	37.4	2.78	2.27
100 fat-free meat powder.	5	2	18.4	8.10	
0	5	2	0.8	3.5	

(Dog 7)

43 sugar.....	7	3	37.2	1.77	3.7
80 fat-free meat powder.	5	3	22.0	5.96	
0	5	3	6.06	2.57	

for that with sugar feeding due to the fact that the dogs did not absorb the meat powder as well as expected. However, judging from the glycosurias of the different regimes, the tissue utilization during the meat feeding period should have been approximately the mean of those of the sugar feeding and of the fasting periods. The tissues of Dog 6 during the sugar feeding period utilized 17.6 grams sugar in addition to that which came from the protein catabolized, and during the fasting period utilized the sugar coming from the protein minus the sugar excreted (0.8 gram). The average of these is 8.3 grams sugar plus 3.14 grams nitrogen. The calculation for the sugar-nitrogen equivalent of the meat period would then be (18.4 plus 8.3) grams sugar which is equivalent to (8.10 — 3.14) grams nitrogen, or an actual D:N ratio of 5.38. The calculation for Dog 7 would be similar (22 — 0.13) grams sugar which is equivalent to (5.96 — 2.17) grams nitrogen or an actual D:N ratio of 5.78.

DISCUSSION

The experimental results given in this paper indicate that the conventional interpretation of the D:N ratio in metabolism and dietetics should be changed. To the urinary sugar excreted by the fasting phlorhizinized dog must be added an amount equal to that which is utilized by the tissues in order to derive the sugar equivalent of protein from the urinary D:N ratio. Our findings show that one is not justified in deducing this

equivalent from the extra sugar excreted by phlorhizinized dogs after being fed protein, since this feeding increases the tissue utilization of glucose. In the fasting phlorhizinized dog the glucose utilization rate of the tissues is 75 mgm. per kilo per hour (10). When this amount is added to that which is excreted in the urine, we obtain a D:N ratio of 6 instead of the conventional 3.65, and a D:Protein ratio close to 1.

Similarly, the tissues of the fasting depancreatized animal utilize sugar (17) and a similar correction should be made here if one wishes to derive the protein-sugar equivalent from observations on this preparation. There is some increase in utilization on feeding glucose (18) but it does not increase as markedly with feeding as in the case of the phlorhizinized fed dog. This is apparent from the fact that the urinary D:N ratios of almost all our protein-fed depancreatized dogs were higher than the usual 2.7 ratio for fasting, and this despite some insulin given to our animals. This fact has made it possible for us to determine the protein-sugar equivalent from our protein-fed depancreatized dogs since here tissue utilization is relatively small and can be corrected for by control periods of sugar feeding. When this is done we find that the true D:N ratio is between 5 and 6.

The highest urinary D:N ratio obtained for fasting depancreatized dogs is 2.7. Even severe diabetic patients rarely show as high ratios as this. The difference between the values for this ratio and that for the true ratio indicates that there is ample glucose available from that converted from body protein to take care of the tissue needs in the fasting diabetic. This can be seen from the following calculations. The normal fasting dog excretes 10.8 mgm. N per kilo per hour (19). If we accept the true D:N ratio as 5.50, this would indicate a tissue utilization of glucose by this animal of 60 mgm. per kilo per hour. Fasting man excretes 7.6 mgm. per kilo per hour (20), which would indicate a tissue utilization of 42 mgm. per kilo per hour. The fasting phlorhizinized dog (4th to 6th day of fasting) excretes 37.5 mgm. N and 136 mgm. glucose per kilo per hour (4). This nitrogen would be equivalent to 37.5×5.5 mgm. or 207 mgm. glucose. Subtracting from this the glucose excreted in the urine,

we obtain a tissue utilization of 70 mgm. per kilo per hour. The fasting depancreatized dog (3rd to 6th day of fasting) excretes 22.3 mgm. N and 65 mgm. glucose per kilo per hour (21). The tissue utilization here would be (22.3×5.5) minus 65, or 58 mgm. glucose per kilo per hour. A very severe case of human diabetes (3rd to 5th day fasting) excreted 19.8 mgm. N and 55 mgm. glucose per kilo per hour (22). This would indicate a tissue utilization of (19.8×5.5) minus 55, or 54 mgm. glucose per kilo per hour. All experimental work leads us to expect about the same tissue utilization for these different conditions (17). If protein is the only non-carbohydrate source of glucose this utilization could only be accounted for by having a much higher conversion ratio than the conventional one.

Supporters of the overproduction theory have had to take recourse to the fat-to-sugar notion in order to account for the large amounts of sugar excreted in the urine of the fasting diabetic in addition to that utilized by his tissues. They have postulated this conversion since they cannot understand how a sufficiently large amount of sugar can come from the protein catabolized to account for the sugar burned plus that which is excreted. We have shown here that conversion of protein to sugar can account for this when one revises the D:N ratio to a higher value and keeps a strict accounting of the amounts involved.

The facts presented in this paper help in giving a satisfactory explanation of the disturbance of carbohydrate metabolism in diabetes. The trouble in diabetes cannot be accounted for by an inability of the tissues to oxidize any glucose; they do utilize definite amounts of this foodstuff (17). The chief fault, as far as carbohydrate metabolism is concerned, appears to be that the body cannot dispose of surplus glucose by changing it to glycogen, fat and other possible glucose derivatives. The amount of glucose oxidized directly by the tissues in the fasting diabetic can be adequately supplied by conversion from body protein, even though considerable amounts of glucose are being excreted in the urine. In this way those well-established facts concerning carbohydrate metabolism in diabetes can be accounted for without having recourse to a conjectural hypothesis involving the conversion of fat to sugar.

SUMMARY

For the classical D:N ratio of 3.65 or D:Protein ratio of 0.58 in the phlorhizinized fasting dog, we must consider the urinary sugar as but part of the total sugar produced from the protein represented by the nitrogen of the urine. The other portion of this sugar is used by the tissues of the animal. On feeding the animal protein, there is a definite increase in this tissue utilization so that the urinary D:N ratio actually decreases.

The depancreatized dog does not show such a marked increase in glucose tissue utilization on feeding. In this preparation one can estimate the extra sugar resulting from fed protein since the amount used by the tissues is small and is easily corrected for. When this correction is applied the D:N ratio is between 5 and 6 and then the D:Protein ratio approaches 1.

With this higher D:N ratio we can account for both the glucose excreted in the urine and that used by the tissues in fasting diabetics as coming from body protein and it is unnecessary to invoke the aid of the questionable notion of conversion of fat-to-glucose.

BIBLIOGRAPHY

1. MacLeod, J. J. R., *Der Brennstoff des Lebens. Ergebn. d. Physiol.*, 1930, 30, 408.
Idem, Diabetes as a physiological problem. *Lancet*, 1930, 2, 383.
2. Reviewed in Joslin, E. P., *Treatment of Diabetes*. Lea and Febiger, Philadelphia, 1937, 6th Ed.
3. Lusk, G., *The Science of Nutrition*. W. B. Saunders, Philadelphia, 1928, 4th Ed.
4. Reilly, F. J., Nolan, F. W., and Lusk, G., Phlorhizin diabetes in dogs. *Am. J. Physiol.*, 1898, 1, 395.
5. Deuel, H. J., Jr., Wilson, H. E. C., and Milhorat, A. T., Animal calorimetry; mechanism. The action of phlorhizin diabetes. *J. Biol. Chem.*, 1927, 74, 265.
6. Wierzechowski, M., Intermediary carbohydrate metabolism; ketosis in phlorhizin diabetes. *J. Biol. Chem.*, 1927, 73, 417.
7. Janney, M. W., and Blatherwick, N. R., Glucose formation from human proteins. *J. Biol. Chem.*, 1915, 23, 77.
8. Gray, J. S., Ivy, A. C., and Cuthbert, F. P., The conversion of protein to glucose in depancreatized and phlorhizinized dogs. *J. Biol. Chem.*, 1939, 128, 173.
9. Bollman, J. L., Mann, F. C., and Magath, T. B., Studies on physiology of liver; effect of total removal of the liver on deamination. *Am. J. Physiol.*, 1926, 78, 258.

10. Drury, D. R., Bergman, H. C., and Greeley, P. O., The glucose utilization of phlorhizinized dogs after hepatectomy. *Am. J. Physiol.*, 1936, 117, 323.
11. Bergman, H. C., and Drury, D. R., Effect of feeding and fasting on sugar utilization of eviscerated rabbits. *Proc. Soc. Exper. Biol. and Med.*, 1937, 37, 414.
12. Hofmeister, F., Ueber Resorption und Assimilation der Nahrstoffe. *Arch. f. exper. Path. u. Pharmacol.*, 1889, 26, 355.
13. Chambers, W. H., Undernutrition and carbohydrate metabolism. *Physiol. Rev.*, 1938, 18, 248.
14. Himwich, H. E., and Nahum, L. H., The respiratory quotient of the brain. *Am. J. Physiol.*, 1932, 101, 446.
15. Yater, W. M., Markowitz, J., and Cahoon, R. F., Consumption of blood sugar by muscle in the non-diabetic and in the diabetic state. *Arch. Int. Med.*, 1933, 51, 800.
16. Lusk, G., *The Science of Nutrition*, W. B. Saunders, Philadelphia, 1928, 4th Ed., p. 79.
- 17a. Greeley, P. O., and Drury, D. R., The glucose utilization of hepatectomized diabetic rabbits. *Am. J. Physiol.*, 1940, 130, 249.
- b. Mann, F. C., and Magath, T. B., The effect of total removal of the liver after pancreatectomy on the blood sugar level. *Arch. Int. Med.*, 1923, 31, 797.
18. Soskin, S., The utilization of carbohydrate by totally depancreatized dogs receiving no insulin. *J. Nutrition*, 1930, 3, 99.
19. Voit, E., Quoted by Lusk. *The Science of Nutrition*, W. B. Saunders, Philadelphia, 1928, 4th Ed., p. 108.
20. Benedict, F. G., *A study of prolonged fasting*. Carnegie Institution of Washington Pub. 203, 1915.
21. Von Falkenhausen, M., Untersuchungen über den Eiweissstoffwechsel beim experimentellen Pankreasdiabetes. *Arch. f. exper. Path. u. Pharmacol.*, 1925, 109, 249.
22. Geyelin, H. B., and Dubois, E. F., A study of blood, urine and respiratory metabolism. *J. A. M. A.*, 1916, 66, 1532.

THE MEASUREMENT AND METABOLISM OF THIAMIN AND OF A PYRIMIDINE STIMULATING YEAST FERMENTATION FOUND IN THE BLOOD CELLS AND URINE OF NORMAL INDIVIDUALS¹

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The methods usually employed at present to ascertain the existence of thiamin deficiency in man are based upon measurements of the amounts of the vitamin in the urine before and after its oral or parenteral administration (1, 2). Evidence is available, however, to indicate that only a fraction of the thiamin ingested in the normal diet is excreted unchanged (3, 4), and hence it is possible that the amount in the urine does not represent the true status of deficiency or sufficiency of the vitamin in the body as a whole. Measurement of the levels of circulating thiamin clearly is preferable, but insufficient information has been available hitherto concerning its distribution between the various constituents of the blood. Due to this fact, the presence of anemia, dehydration, or marked leukocytosis might provide misleading findings if only the levels in whole blood were employed.

No method has been at hand for the measurement of the minute amounts of thiamin contained in the cellular elements since neither the technique of Melnick (5), nor the thiochrome method of Hennessy (6) are sensitive enough to be used for this purpose. It has been possible, however, so to adapt the ultramicro-technique of Atkin, Schultz and Frey (7) that thiamin can be determined in either leukocytes or erythrocytes. The method was chosen, not only because of its sensitivity and accuracy, but also because it made possible the measurement of both thiamin and the pyrimidines capable of accelerating yeast fermentation (PAYF).³

From the similarity between the chemical struc-

tures of thiamin and the pyrimidine compounds known to accelerate yeast fermentation (8), it has been assumed that the latter substances are either precursors of thiamin or products of its breakdown. On the basis of this possibility the simultaneous measurement of both thiamin and the PAYF compound in blood cells and urine, after the administration of those substances, should provide new information concerning the metabolism of thiamin by the human organism. Experiments of this type have been published by Pollack *et al.* (4). They found that the parenteral administration of 100 mgm. of thiamin resulted in an increased urinary excretion of pyrimidine and concluded that in the normal individual pyrimidine is derived from thiamin.

This communication deals with (1) a method for the measurement of the thiamin and the PAYF content of blood cells and urine; (2) the amounts of thiamin and the PAYF compound in leukocytes and platelets, erythrocytes, and the urine of normal individuals; and (3) a study of the metabolism of thiamin by the normal human being.

METHOD

The techniques used for the quantitative determination of total thiamin and of the pyrimidine compound in the blood and urine are adaptations of the fermentation method of Schultz, Atkin and Frey (7, 8). They depend upon the principle that within certain limits both thiamin and PAYF cause a measurable increase in the rate of alcoholic fermentation by yeast of a suitable sugar-salt-buffer mixture.

In this communication the term "total thiamin" is employed to include all those substances capable of stimulating fermentation by yeast under the conditions of the experimental procedure.

A. Determination of the total thiamin and PAYF in blood cells

To obtain leukocytes and platelets, 25 ml. of oxalated blood are allowed to settle at room temperature for from

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³ Pyrimidine accelerating yeast fermentation.

It would appear, therefore, that leukocyte fragmentation does not entail a serious loss of total thiamin. It is probable that the thiamin is bound firmly to the solid insoluble matter of the white cells. This supposition is in accord with the findings of Banga, Ochoa, and Peters (10) who observed that no thiamin pyrophosphate could be separated from brain even when that tissue is ground up or heated to 100° C.

c. The total thiamin content of platelets and leukocytes obtained from the same suspension of white cells. It has been indicated that the suspension of white cells obtained for their thiamin measurement consists of a mixture of platelets and leukocytes. Therefore, before it was permissible to compare the concentration of total thiamin in one mixture of white cell elements with that of another, it was necessary first to determine whether or not any significant difference existed between the concentrations of total thiamin in the leukocytes and in the platelets obtained from the same suspension of white cells.

By the refined technique previously described it was possible to separate from the white cell suspensions two fractions: one of pure platelets and the other of leukocytes with a small amount of platelets. Microscopic examination revealed that the latter fraction contained a concentration of leukocytes from 3 to 4 times that in the original white cell suspension, and for this experiment this fraction was considered to be one of leukocytes alone.

The data in Table III present two experiments in which total thiamin values were determined in equal volumes of platelets and of leukocytes obtained from the same sample of white cells. In the two experiments it was found that the thiamin contents of platelets were only 10 and 11 per cent less than those in the corresponding leukocytes.

It would appear, therefore, that since the total thiamin contents of leukocytes and platelets ob-

TABLE III

The thiamin content of platelets and leukocytes partitioned from the same samples of white cells

Individual	Total thiamin in	
	Leukocytes	Platelets
	<i>micrograms per 100 ml.</i>	
H. R.....	127	113
S. K.....	190	170

tained from the same samples of white cells are approximately equal, it is permissible to compare the concentrations of thiamin in one sample of white cells with that of another.

d. Recovery of thiamin and of PAYF added to whole blood, blood cell suspensions, and to urine. The data in Table IV present determinations

TABLE IV
Recovery of thiamin from whole blood and urine

	Millimicrograms of thiamin added to 100 ml. of sample	Millimicrograms of thiamin in 100 ml. of sample	Per cent of recovery
Blood number 1	0 4	8.9 13.6	105
Blood number 2	0 6 10	3.6 9.0 14.2	94 104
Blood number 3	0 2 4 6	10.4 12.0 14.0 16.2	97 97 99
Blood number 4	0 4 6 8	5.4 9.5 9.2 12.7	102 80 91
Blood number 5	0 10 20	4.0 15.8 26.5	111.4 109.4
Urine number 1	0 25	36.25 61.25	100
Urine number 2	0 50	3.75 52.50	97.5
Urine number 3	0 50	50.0 88.75	88.75

of total thiamin in 5 samples of blood and in 3 samples of urine before and after known amounts of thiamin had been added. The recovery of the vitamin added to the blood ranged from 80.0 per cent to 111.4 per cent, and of that added to the urine from 88.75 per cent to 100.0 per cent.

Table V presents the amounts recovered from samples of white cells, of erythrocytes and of urine to which varying amounts of 2-methyl-5-methoxyethyl-6-amino-pyrimidine, a known accelerator of yeast fermentation (8), had been added. The recoveries of that substance added to the blood cell suspensions ranged from 93 per cent to 105 per cent, and of that added to urine from 92.8 per cent to 96.5 per cent.

TABLE V

Recovery of 2-methyl-5-methoxyethyl-6-amino-pyrimidine from blood cells and urine

Sample	Micro-grams* of PAYF found in 100 ml. of sample	Micro-grams* of 6-amino-pyrimidine added to 100 ml. of sample	Micro-grams* of PAYF recovered	Per cent of recovery
White blood cells number 1...	3.2	20	22.8	93
White blood cells number 2...	3.4	20	24.4	105
White blood cells number 3...	1.3	20	21.4	101
Red blood cells number 1....	2.9	20	24.2	105
Red blood cells number 2....	3.3	20	23.4	101
Urine number 1.....	4.5	50	52.5	94.5
Urine number 2.....	44.5	25	64.5	92.0
Urine number 3.....	22.5	50	70.0	96.5

* Micrograms in thiamin equivalents.

These results indicate that the methods employed satisfactorily measure thiamin and the pyrimidine compound in blood and urine specimens.

II. The concentration of total thiamin and PAYF in the blood cells and urine of normal individuals

Determinations were made of the total thiamin in the white cells of 30 normal adults—18 females and 12 males—and in the erythrocytes of 24 of those same individuals—17 females and 7 males. The total thiamin of the white cells ranged from 48 to 183 micrograms per 100 ml. and averaged 99.8 micrograms per 100 ml. The total thiamin of the erythrocytes ranged from 3.7 to 38.0 micrograms per 100 ml. and averaged 10.3 micrograms per 100 ml. (Table VI, Figure 2).

The levels of PAYF were measured in the white cells of 10 of the 30 normal individuals studied, and in the red cells of 6 of the 24. The concentrations of the PAYF compound in the white cells ranged from 11 to 50 micrograms per 100 ml. and averaged 32.5 micrograms per 100 ml. The PAYF in these 10 instances accounted for from 16.8 per cent to 64 per cent of the total white cell thiamin measured. In the erythrocytes the PAYF concentrations ranged from 1.3 to 5.0 micrograms per 100 ml. and averaged 3.0 micrograms per 100 ml. The PAYF accounted for from 14 per cent to 30 per cent of the total red cell thiamin.

The daily excretion of total thiamin and PAYF in the urine was followed in 8 normal individuals

TABLE VI

The concentrations of total thiamin, true thiamin and of PAYF in the blood cells of normal individuals

Individual	Total thiamin		True thiamin		PAYF	
	White blood cells	Red blood cells	White blood cells	Red blood cells	White blood cells	Red blood cells
	micrograms per 100 ml.		micrograms per 100 ml.		micrograms* per 100 ml.	
1 KS	85	11.0				
2 JS	92.5	4.5				
3 LM	89.5	3.7				
4 MR	49.0	20.5				
5 NY	183	14.8				
6 MB	150	10.9				
7 AG	48	5.1				
8 AD	107.5	31.6				
9 JA	64.5	7.2				
10 VW	49	14.2				
11 MS	119	8.5				
12 MD	56	4.9				
13 CH	105	9.0				
14 JL	105	4.1				
15 PH	156	10.8				
16 NH	89.5	5.5				
17 AR	76	4.7				
18 HS	124	10.7				
19 JP	57					
20 WB	180					
21 AB	143	15.6	100	11.0	43	4.5
22 JH	107	16.5	81	14.2	26	2.3
23 MH	108	8.0	58	5.0	50	3.0
24 AS	80	15.0	69	10.0	11	5.0
25 PR	114	7.7	70	6.4	44	1.3
26 MP	110	7.0	70	5.0	40	2.0
27 DT	74		54		20	
28 MD	58		21		37	
29 RJ	66		50		16	
30 SE	171		133		38	
Average	99.8	10.3	70.6	8.6	32.5	3.0

* Micrograms expressed in thiamin equivalents.

TABLE VII

Micrograms of thiamin and of PAYF excreted per day in the urine of normal individuals

Subject	Weight	Number of days	Urinary thiamin	Average per day	Highest per cent daily variation	Urinary PAYF*	Average per day	Highest per cent daily variation
			Range per day			Range per day		
J.J.	kilos	9	76-227	140	200	294-525	368	78
J.H.	82	6	179-421	350	135	351-910	556	159
M.P.	69	6	53-168	111	216	126-282	198	124
N.N.	53	4	140-348	270	143	215-300	263	40
M.H.	45	4	66-101	83	53	300-580	426	93
A.B.	61	2	25-63	44	152	169-220	194	35
P.R.	83	4	158-314	214	99	408-514	460	41
A.S.	42	3	158-296	229	87	222-332	250	50

* Micrograms expressed in thiamin equivalents.

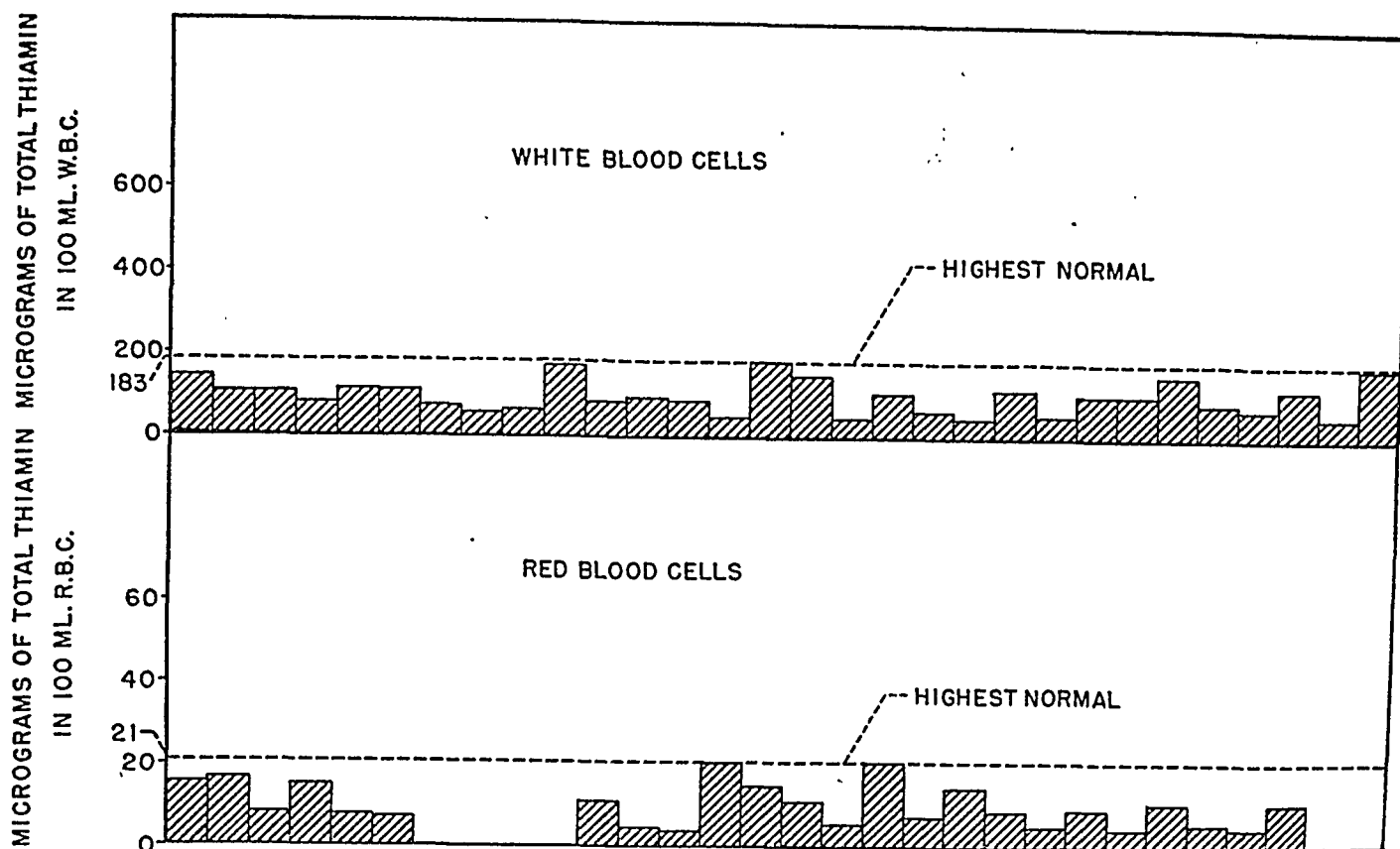


FIG. 2. THE LEVELS OF TOTAL THIAMIN IN THE BLOOD CELLS OF NORMAL INDIVIDUALS

for periods of from 2 to 9 days. In all, 45 determinations were made. The average of the true thiamin excretion of each of 8 individuals ranged from 44 to 229 micrograms per day, and the average of the group was 168 micrograms per day. These values are in good agreement with those obtained by Schultz, Light and Frey (11). The average of the PAYF excretions of each of the 8 normals ranged from 194.5 to 556 micrograms per day, and the average of the group was 340 micrograms per day (Table VII). The highest percentage of daily variations of the thiamin excretion in the urine of these individuals was from 87 to 216 per cent, and that of the PAYF from 35 to 159 per cent.

III. The correlation between the total thiamin levels in the blood cells and the ingestion of thiamin

To ascertain if the blood cell total thiamin levels closely reflect the thiamin deficiency or saturation of the body, determinations were made in the cells of individuals (a) who had clinical evidence of thiamin deficiency, and (b) who had received supplements of the vitamin added to their diet.

a. The blood cell total thiamin of patients with clinical evidence of thiamin deficiency. Table VIII presents analyses of total thiamin in the white cells and red cells of 4 individuals who had evidence of vitamin B₁ deficiency as ascertained

TABLE VIII
The concentrations of total thiamin in the blood cells of patients with clinical evidence of thiamin deficiency

Patient	Diagnosis	Symptoms	Total thiamin	
			White blood cells	Red blood cells
			<i>micrograms per 100 ml.</i>	
JS	Ulcerative colitis	Anorexia Diarrhea Paresthesias	34.5	3.9
MS	Pyloric ulcer with obstruction	Polyneuritis Absent knee jerks Vomiting Anorexia	32.9	2.0
MR	Cirrhosis of liver, alcoholism	Peripheral edema Absent knee jerks Right ankle drop	21.0	1.7
BL	Chronic inanition	Marked anorexia Weight loss General inhibition Paresthesias	16.7	4.1

from dietary history and physical examination. Urinary thiamin measurements were not being made when these patients were available.

The average white cell total thiamin of this group was 26.3 micrograms per 100 ml., or about 26 per cent of the normal value. The average red cell thiamin was 2.9 micrograms per 100 ml., or about 29 per cent of the normal.

b. *The blood cell total thiamin of individuals who had ingested excess amounts of the vitamin.* Table IX presents the values for total thiamin in the blood cells of 5 normal individuals who had been given daily doses of 10 mgm. of the crystalline vitamin orally for from 3 to 7 days.

All blood specimens of this group were taken from 20 to 24 hours after the last dose of thiamin was administered.

TABLE IX

The concentrations of total thiamin in the blood cells of individuals who received daily 10 mgm. supplements of thiamin

Subject	Total thiamin	
	White blood cells	Red blood cells
	<i>micrograms per 100 ml.</i>	
RB.....	216	53.5
WS.....	244	38.5
JP.....	292	18.7
NL.....	202	16.7
MM.....	399	16.3

The average blood cell total thiamin levels of this group were 270.6 micrograms per 100 ml. of white cells and 28.8 micrograms per 100 ml. of erythrocytes. These average levels are about 3 times those found in the white and red cells of normal individuals who had not received supplements of the vitamin.

These high levels of total thiamin in the leukocytes and platelets of patients fed 10 mgm. of thiamin daily indicate that those cells normally are not saturated with the vitamin. If the administration of large amounts of thiamin to normal individuals only elevates their white cell levels of the vitamin and does not bring those levels all within the same narrow range, it would appear that normal white cells differ considerably in their capacity to absorb the vitamin. On the other hand, if it were demonstrated that the white cells of normal individuals can absorb only a limited and maximum amount of thiamin, despite the amounts of the vitamin supplements ingested, then low levels of thiamin in the leukocytes and

platelets under normal conditions would indicate subclinical thiamin deficiency.

To ascertain the facts concerning this question, the total thiamin levels in the white cells of 8 normal individuals were determined before and, in 6 of the 8, after the daily administration of 100 mgm. of thiamin for 6 days (Table X).

TABLE X

Micrograms of total thiamin in 100 ml. of white cells of individuals who received daily 100 mgm. supplements of thiamin

Subject	Before administration of thiamin	1 Day after oral administration of 600 mgm. of thiamin	8 Days after discontinuing oral administration of thiamin
N. J.	66	264	67
J. P.	57	263	100
A. B.	166	222	100
D. T.	74	207	86
M. D.	56	225	52
S. E.	171	210	
J. H.	94	208	
W. B.	180	235	113
Average	108	229	86

These 8 subjects had been taking apparently normal diets and no supplements of the vitamin for several weeks. The pre-therapeutic levels of total thiamin in their white cells ranged from 56 to 180 micrograms per 100 ml. and averaged 108 micrograms. After the daily ingestion of 100 milligrams of the vitamin for 6 days, the levels of total thiamin in those cells ranged from 207 to 265 micrograms per 100 ml. and averaged 229 micrograms, or twice the pre-therapeutic level. These increased levels are in the same range as those found in the leukocytes and platelets of 4 of the 5 patients who received only 10 milligrams of thiamin each day for 1 week. Finally, 8 days after the daily feeding of the 100 milligram doses of the vitamin had been discontinued, the white cell levels of total thiamin of 6 of the 8 subjects had fallen again to from 52 to 113 micrograms per 100 ml. This post-therapeutic level was not determined in the white cells of the other 2 subjects.

The fact is to be emphasized that, whereas the variation of total thiamin in the white cells of individuals before their ingestion of excess amounts of the vitamin is as high as 220 per cent, the variation after the thiamin administration is only 28 per cent. Hence, it might be concluded that white cells do not differ widely in their

capacity to absorb thiamin, but can absorb only a limited amount of the substance. This fact would suggest, therefore, that individuals who are taking an apparently normal diet but who have low leukocyte and platelet levels of total thiamin possibly are in a clinical or subclinical deficiency state as regards vitamin B₁.

The intravenous administration of 1 mgm. of crystalline thiamin to 2 normal individuals also raised the levels of total thiamin in the leukocytes and platelets. In the case of N. Y., the white cell total thiamin level promptly rose from 140 micrograms to 850 micrograms per 100 ml. within 1 hour, but gradually returned to its original value by the next day. The white cell total thiamin level of M. R. also rose from 49 to 160 micrograms per 100 ml. of cells within 1 hour after the thiamin injection and returned to its original level by the next day. No significant changes were observed in the levels of total thiamin of the erythrocytes of these 2 individuals after the 1 mgm. dose.

Therefore, from the evidence presented, it may be concluded that the level of total thiamin in the white cells, and perhaps in the erythrocytes, is an index of the degree of thiamin saturation of the organism.

IV. The metabolism of thiamin by the normal individuals

The thiamin molecule is composed of two nuclei: the pyrimidine and the thiazol (Figure 1). The fact has been established that yeast and other forms of plant life can synthesize thiamin from the derivatives of pyrimidine which hydrolyze in solution to form 2-methyl-5-hydroxymethyl-6-amino-pyrimidine (12).

The vitamin also is broken down by these micro-organisms with the destruction of the thiazol ring and liberation of the free pyrimidine (13). No such mechanism has been proved to occur in the animal. Robbins *et al.* (14) found that, while the polyneuritis of pigeons could be cured by feeding a mixture of pyrimidine and thiazol, the amounts needed were several thousand times that of an equivalent and curative amount of thiamin. They concluded, therefore, that pigeons had only a slight capacity for the synthesis of the vitamin from its two nuclei. In the human it is unknown whether the pyrimidine compound is a precursor or a break-

down product of thiamin, or, indeed, if it bears any metabolic relationship to the vitamin at all.

In their study of the specificity of thiamin in the acceleration of yeast fermentation, Schultz, Atkin and Frey (8) tested numerous pyrimidine derivatives. They were able to show that of all the pyrimidine compounds tested for thiamin-like, yeast-stimulating activity, only 2-methyl-5-hydroxymethyl-6-amino-pyrimidine, or compounds which in solution conceivably could hydrolyze to form that substance, possessed such activity. As previously indicated, compounds which could hydrolyze to form this hydroxy derivative do not form a sulfonate with the sulfite used to cleave the thiamin, but remain free to accelerate the fermentation. On the other hand, the pyrimidine nucleus which is split from the thiazol by sulfite is converted immediately into a sulfonate which lacks the yeast-stimulating activity.

Since a sensitive and accurate method was at hand for the measurements of thiamin and the active PAYF, it was possible to investigate the relationship in man between these two compounds. The existence in the blood and urine of two substances with similar chemical structure suggested that the PAYF is either a precursor, or, more likely, in view of Robbin's experiments (14), a metabolite of thiamin. Two other possibilities, however, existed: that either thiamin or the pyrimidine might be converted to the other, or that there is no metabolic relationship between the two substances.

It was thought that the facts concerning this question could be ascertained by the administration of test doses of thiamin and of a pyrimidine compound known to be an active accelerator of yeast fermentation, and then by measurement of the changes effected in the concentrations of these substances in the white cells and urine. Two pyrimidines were available for this purpose: one, the synthetic 2-methyl-5-methoxyethyl-6-amino-pyrimidine (8), and the other, the pyrimidine formed by the alkaline cleavage of thiamin.⁵

⁵ The latter substance was prepared by Dr. L. Atkin of the Fleischmann Laboratories by heating at 115° for 4 hours a solution of thiamin at pH 6.4. After the thiamin molecule was cleaved, the solution was acidified to pH 5. This solution finally contained 576 micrograms per ml. of an active pyrimidine (expressed in thiamin equivalents) and 67 micrograms per ml. of thiamin.

If thiamin is converted during the course of its activity into an active pyrimidine compound, then the administration of the vitamin should increase the white cell content both of thiamin and of PAYF. The administration of the pyrimidine compound, on the other hand, should increase only the white cell content of PAYF. Conversely, if the PAYF is a precursor of thiamin, then the administration of the pyrimidine should increase both the PAYF and thiamin of the white cells, but the administration of thiamin should be followed only by an increased white cell thiamin.

The study of the metabolism of thiamin by the blood cells of human beings was made on 5 normal adults, 3 females and 2 males. All were on satisfactory diets, without supplements of vitamin B₁. The concentrations of true thiamin and of the PAYF in the white cells, erythrocytes and urine were determined for each subject 12 hours after the last ingestion of food. Then each was given intravenously 5 mgm. of thiamin and several days later, under the same conditions, 5 mgm. of an active pyrimidine. Determinations of the levels of true thiamin and of the PAYF in the blood cells were made, 1, 3, and 24 hours after each injection (Table XI, Figures 3, 4). In addition, the concentrations of true thiamin and PAYF were ascertained in the urine of 9 normal individuals collected through the 1st hour, from the 1st to the 3rd, and from the 3rd to the 24th hour after the administration of the test substances. All the blood and the first 2 urine specimens were obtained when the subject was in the fasting state.

The levels of true thiamin and the PAYF in the white cells, rather than those in the erythrocytes, were used as an index of the absorption of those substances by the body, because earlier experiments (cited in Section IIIb) indicated that the intravenous administration of 1 mgm. doses of the vitamin to normal individuals produced a marked rise in the white cell total thiamin level but no significant changes in the red cell level.

a. *Results after the intravenous administration of 5 mgm. of thiamin.* The intravenous administration of 5 mgm. of thiamin to the normal subjects was followed within 3 hours by increases in the levels of true thiamin in the white cells (Figure 3). These levels ranged from 80 per cent to 132 per cent above their original values. The

average increase was 100 per cent. In all instances, the administration of thiamin also produced within the first 3 hours significant increases in the white cell levels of PAYF. The post-treatment levels ranged from 41 per cent to 180

TABLE XI

The concentrations of true thiamin and of PAYF in the white cells and erythrocytes of normal individuals given thiamin and 2-methyl-5-methoxyethyl-6-amino-pyrimidine*

Subject	Injection	Hours after injection	White blood cells		Red blood cells	
			True thia-min	PAYF	True thia-min	PAYF
			micrograms per 100 ml.		micrograms per 100 ml.	
J. H.	5 mgm. B ₁	0	81	26	14.2	2.3
		1	155	35	11.5	2.3
		3	78	71	8.6	9.7
		24	90	26	13.5	2.6
	5 mgm. 6AP	0	68	97	11.2	2.4
		1	66	130	0	18.0
		3	84	47	13.0	2.4
		24	45	32	13.1	4.3
	5 mgm. B ₁	0	50	16	6.3	1.1
		1	105	40	5.0	2.1
		3	95	25	12.0	5.6
		24	56	15	7.6	2.2
P. R.	5 mgm. 6AP	0	70	44	6.4	1.3
		1	30	50	8.1	5.7
		3	63	194	15.0	15
		24	330	70	0	10
A. B.	5 mgm. B ₁	0	100	43	11.1	4.5
		1	232	42	7.7	4.4
		3	32.5	98.5	11.8	4.5
		24	66.5	33.5	8.2	4.0
	5 mgm. 6AP	0	100	43	7.2	3.0
		1	66	80	9.2	11.4
		3	96.5	71.5	13.6	3.4
		24	131	40	1.2	3.3
M. H.	5 mgm. B ₁	0	50	29	3.0	7.0
		1	89	41	8.7	5.5
		3	77	40	8.4	4.2
		24	369	13	5.3	1.1
	5 mgm. 6AP	0	58	50	3.2	5.0
		1	33	235	0	16.1
		3	83	59	7.1	7.2
		24	90	10	9.0	5.0
A. S.	5 mgm. B ₁	0	165	31	12.5	2.1
		1	236	61	11.7	3.8
		3	236	40	3.2	4.5
		24	148	8	9.7	3.1
	5 mgm. 6AP	0	69	11	10.0	5.0
		1	53	67	7.4	11.1
		3	52	45	8.5	1.5
		24	106	34	3.7	10.0

* PAYF expressed in micrograms of thiamin equivalents.

capacity to absorb thiamin, but can absorb only a limited amount of the substance. This fact would suggest, therefore, that individuals who are taking an apparently normal diet but who have low leukocyte and platelet levels of total thiamin possibly are in a clinical or subclinical deficiency state as regards vitamin B₁.

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Since a sensitive and accurate method was at hand for the measurements of thiamin and the active PAYF, it was possible to investigate the relationship in man between these two compounds. The existence in the blood and urine of two substances with similar chemical structure suggested that the PAYF is either a precursor, or, more likely, in view of Robbin's experiments (14), a metabolite of thiamin. Two other possibilities, however, existed: that either thiamin or the pyrimidine might be converted to the other, or that there is no metabolic relationship between the two substances.

It was thought that the facts concerning this question could be ascertained by the administration of test doses of thiamin and of a pyrimidine compound known to be an active accelerator of yeast fermentation, and then by measurement of the changes effected in the concentrations of these substances in the white cells and urine. Two pyrimidines were available for this purpose: one, the synthetic 2-methyl-5-methoxyethyl-6-amino-pyrimidine (8), and the other, the pyrimidine formed by the alkaline cleavage of thiamin.⁵

⁵ The latter substance was prepared by Dr. L. Atkin of the Fleischmann Laboratories by heating at 115° for 4 hours a solution of thiamin at pH 6.4. After the thiamin molecule was cleaved, the solution was acidified to pH 5. This solution finally contained 576 micrograms per ml. of an active pyrimidine (expressed in thiamin equivalents) and 67 micrograms per ml. of thiamin.

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The study of the metabolism of thiamin by the blood cells of human beings was made on 5 normal adults, 3 females and 2 males. All were on satisfactory diets, without supplements of vitamin B₁. The concentrations of true thiamin and of the PAYF in the white cells, erythrocytes and urine were determined for each subject 12 hours after the last ingestion of food. Then each was given intravenously 5 mgm. of thiamin and several days later, under the same conditions, 5 mgm. of an active pyrimidine. Determinations of the levels of true thiamin and of the PAYF in the blood cells were made, 1, 3, and 24 hours after each injection (Table XI, Figures 3, 4). In addition, the concentrations of true thiamin and PAYF were ascertained in the urine of 9 normal individuals collected through the 1st hour, from the 1st to the 3rd, and from the 3rd to the 24th hour after the administration of the test substances. All the blood and the first 2 urine specimens were obtained when the subject was in the fasting state.

The levels of true thiamin and the PAYF in the white cells, rather than those in the erythrocytes, were used as an index of the absorption of those substances by the body, because earlier experiments (cited in Section IIb) indicated that the intravenous administration of 1 mgm. doses of the vitamin to normal individuals produced a marked rise in the white cell total thiamin level but no significant changes in the red cell level.

a. Results after the intravenous administration of 5 mgm. of thiamin. The intravenous administration of 5 mgm. of thiamin to the normal subjects was followed within 3 hours by increases in the levels of true thiamin in the white cells (Figure 3). These levels ranged from 80 per cent to 132 per cent above their original values. The

average increase was 100 per cent. In all instances, the administration of thiamin also produced within the first 3 hours significant increases in the white cell levels of PAYF. The post-treatment levels ranged from 41 per cent to 180

TABLE XI

The concentrations of true thiamin and of PAYF in the white cells and erythrocytes of normal individuals given thiamin and 2-methyl-5-methoxyethyl-6-amino-pyrimidine*

Subject	Injection	Hours after injection	White blood cells		Red blood cells	
			True thiamin	PAYF	True thiamin	PAYF
			micrograms per 100 ml.		micrograms per 100 ml.	
J. H.	5 mgm. B ₁	0	81	26	14.2	2.3
		1	155	35	11.5	2.3
		3	78	71	8.6	9.7
		24	90	26	13.5	2.6
	5 mgm. 6AP	0	68	97	11.2	2.4
		1	66	130	0	18.0
		3	84	47	13.0	2.4
		24	45	32	13.1	4.3
	5 mgm. B ₁	0	50	16	6.3	1.1
		1	105	40	5.0	2.1
		3	95	25	12.0	5.6
		24	56	15	7.6	2.2
P. R.	5 mgm. 6AP	0	70	44	6.4	1.3
		1	30	50	8.1	5.7
		3	63	194	15.0	15
		24	330	70	0	10
	5 mgm. B ₁	0	100	43	11.1	4.5
		1	232	42	7.7	4.4
		3	32.5	98.5	11.8	4.5
		24	66.5	33.5	8.2	4.0
	5 mgm. 6AP	0	100	43	7.2	3.0
		1	66	80	9.2	11.4
		3	96.5	71.5	13.6	3.4
		24	131	40	1.2	3.3
A. B.	5 mgm. B ₁	0	50	29	3.0	7.0
		1	89	41	8.7	5.5
		3	77	40	8.4	4.2
		24	369	13	5.3	1.1
	5 mgm. 6AP	0	58	50	3.2	5.0
		1	33	235	0	16.1
		3	83	59	7.1	7.2
		24	90	10	9.0	5.0
	5 mgm. B ₁	0	165	31	12.5	2.1
		1	236	61	11.7	3.8
		3	236	40	3.2	4.5
		24	148	8	9.7	3.1
A. S.	5 mgm. 6AP	0	69	11	10.0	5.0
		1	53	67	7.4	11.1
		3	52	45	8.5	1.5
		24	106	34	3.7	10.0

* PAYF expressed in micrograms of thiamin equivalents.

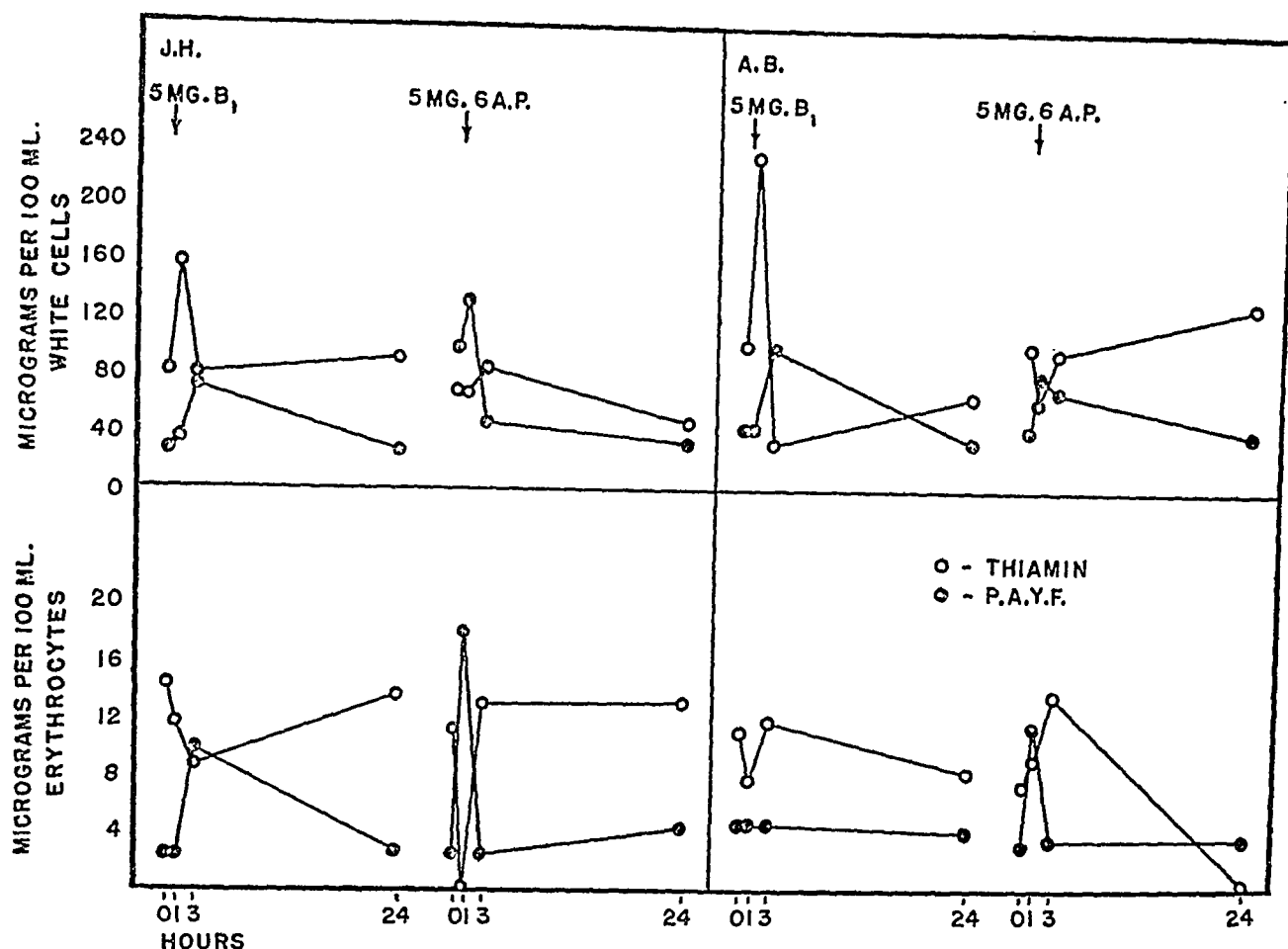


FIG. 3. THE LEVELS OF THIAMIN AND OF PAYF IN WHITE CELLS AND ERYTHROCYTES OF NORMAL INDIVIDUALS AFTER THE INTRAVENOUS ADMINISTRATION OF MG. OF THIAMIN AND 5 MG. OF 2-METHYL-5-METHOXYETHYL-6-AMINO-PYRIMIDINE

per cent of the original value, and averaged 143 per cent.

The concentrations of both true thiamin and the PAYF in the white cells tended to return to their base levels within 24 hours. Only in the subject M. H. did the thiamin level at 24 hours continue to rise.

The levels of true thiamin and the PAYF in the erythrocytes changed in a much less consistent manner after the administration of the vitamin. The erythrocyte true thiamin level rose in only 2 of the 5 subjects, and the PAYF in 3.

The urinary excretions of true thiamin and of the PAYF during the next 24 hours were increased significantly in all of 8 subjects who received injections of the vitamin (Tables XII, XIII, Figure 4). The true thiamin outputs in the 24 hours after the injection ranged from 51 per cent to 1610 per cent above the outputs of the previous day and from 121 per cent to 835 per cent above the average of the true thiamin ex-

creted during all of the control days. The excretions of PAYF ranged from 52 per cent to 276 per cent above those of the previous day, and from 89

TABLE XII

Micrograms of thiamin excreted per day in the urine of individuals before and after the intravenous injection of thiamin

Subject	Control period				After injection		
	Number of days	Urinary thiamin range per day	Average per day	Highest per cent daily variation	24-hour output	Per cent increase over	
						Pre-vious day	Average control day
J. J.	9	76-227	140	200	760	538	442
J. H.	6	179-421	235	135	1530	300	551
M. P.	6	53-168	111	216	714	1249	543
N. N.	5	140-348	270	143	1465	991	442
M. H.	4	66-101	83	53	726	1000	775
A. B.	2	25-63	44	152	411	1610	835
P. R.	4	158-314	214	99	474	51	121
A. S.	3	158-296	229	87	859	190	275

TABLE XIII

Micrograms of PAYF excreted per day in the urine of individuals before and after the intravenous injection of thiamin*

Subject	Control period				After injection		
	Number of days	Urinary PAYF range per day	Average per day	Highest per cent daily variation	24-hour output	Per cent increase over	
						Pre-vious day	Average control day
J. J.	9	294-525	368	78	1280	276	248
J. H.	6	351-910	556	159	2930	222	428
M. P.	6	126-282	198	124	561	198	183
N. N.	5	215-300	263	40	805	258	207
M. H.	4	300-580	426	93	862	187	102
A. B.	2	169-220	194	35	479	116	147
P. R.	4	408-574	460	41	870	52	89
A. S.	3	222-332	250	50	530	60	112

* Expressed in micrograms of thiamin equivalents.

per cent to 428 per cent above the average PAYF excretions of all the control days. In each subject the injection of thiamin resulted in increased outputs of both thiamin and of pyrimidine. These were too great to be explained by the normal daily variation.

From the evidence presented, therefore, it would appear that the intravenous injection of thiamin into normal individuals is followed consistently by significantly increased white cell and urinary concentrations of thiamin and of PAYF.

b. Results of the intravenous administration of the active pyrimidine compounds. The administration of 5 mgm.⁶ of 2-methyl-5-methoxyethyl-6-amino-pyrimidine to 5 normal individuals increased within the first 3 hours their white cell concentrations of PAYF from 34 per cent to 660 per cent above the base levels (Figure 3). The average increase of the white cell level of PAYF was 333 per cent. The level of true thiamin in the white cells was elevated in only 1 subject (M. H.) 3 hours after the injection, but in 3 subjects 24 hours after the injection. In the erythrocytes the injection of the synthetic 6-amino-pyrimidine was followed by increased concentrations of the PAYF which ranged from 220 per cent to 1000 per cent; the average increase was 492 per cent. In 3 individuals the true thiamin levels of the red cells also were increased significantly, from 88

⁶ This amount of 2-methyl-5-methoxyethyl-6-amino-pyrimidine is equivalent to 10.6 mgm. of thiamin.

per cent to 130 per cent and the levels of both true thiamin and of PAYF did not return in all instances to their base levels 24 hours after the injection of the 6-amino-pyrimidine.

The intravenous administration of 5 mgm. of 2-methyl-5-methoxyethyl-6-amino-pyrimidine to 5 normal individuals increased the urinary excretion of the PAYF during the next 24 hours in 4 of the 5 instances from 20 per cent to 127 per cent. The average increase was 72 per cent (Figure 4, Table XIV). The excretion of PAYF by the fifth individual in the 24-hour test period was 20 per cent less than that of his average excretion. It is interesting to note that the urinary output of PAYF after the injection of 5 mgm. of 2-methyl-5-methoxyethyl-6-amino-pyrimidine is considerably

TABLE XIV

Micrograms of PAYF excreted per day in the urine of individuals before and after the intravenous injection of 2-methyl-5-methoxyethyl-6-amino-pyrimidine*

Subject	Control period				After injection		
	Number of days	Urinary PAYF range per day	Average per day	Highest per cent daily variation	24-hour output	Per cent increase over	
						Pre-vious day	Average control day
M. H.	4	300-580	426	93	408	11	-20
A. B.	2	169-220	194	35	356	13	84
P. R.	4	408-514	460	41	553	18	20
A. S.	3	222-332	250	50	568	156	127
J. H.	6	351-910	556	159	880	43	58

* Expressed in thiamin equivalents.

less than the urinary output of PAYF after the injection of 5 mgm. of thiamin (Table XIII).

This observation suggested the possibility that the form in which the pyrimidine degradation product of thiamin occurs in the body is not the 2-methyl-5-methoxyethyl-6-amino-pyrimidine. Therefore, it seemed advisable to inject another active pyrimidine which probably bore a closer relationship to thiamin degradation than did the synthetic 2-methyl-5-methoxyethyl-6-amino derivative. The pyrimidine selected was that formed by the alkaline cleavage of the vitamin. This compound was suggested and prepared by the Fleischmann Laboratories and, of that preparation, amounts equivalent to 5 mgm. of 2-methyl-5-methoxyethyl-6-amino-pyrimidine were given in-

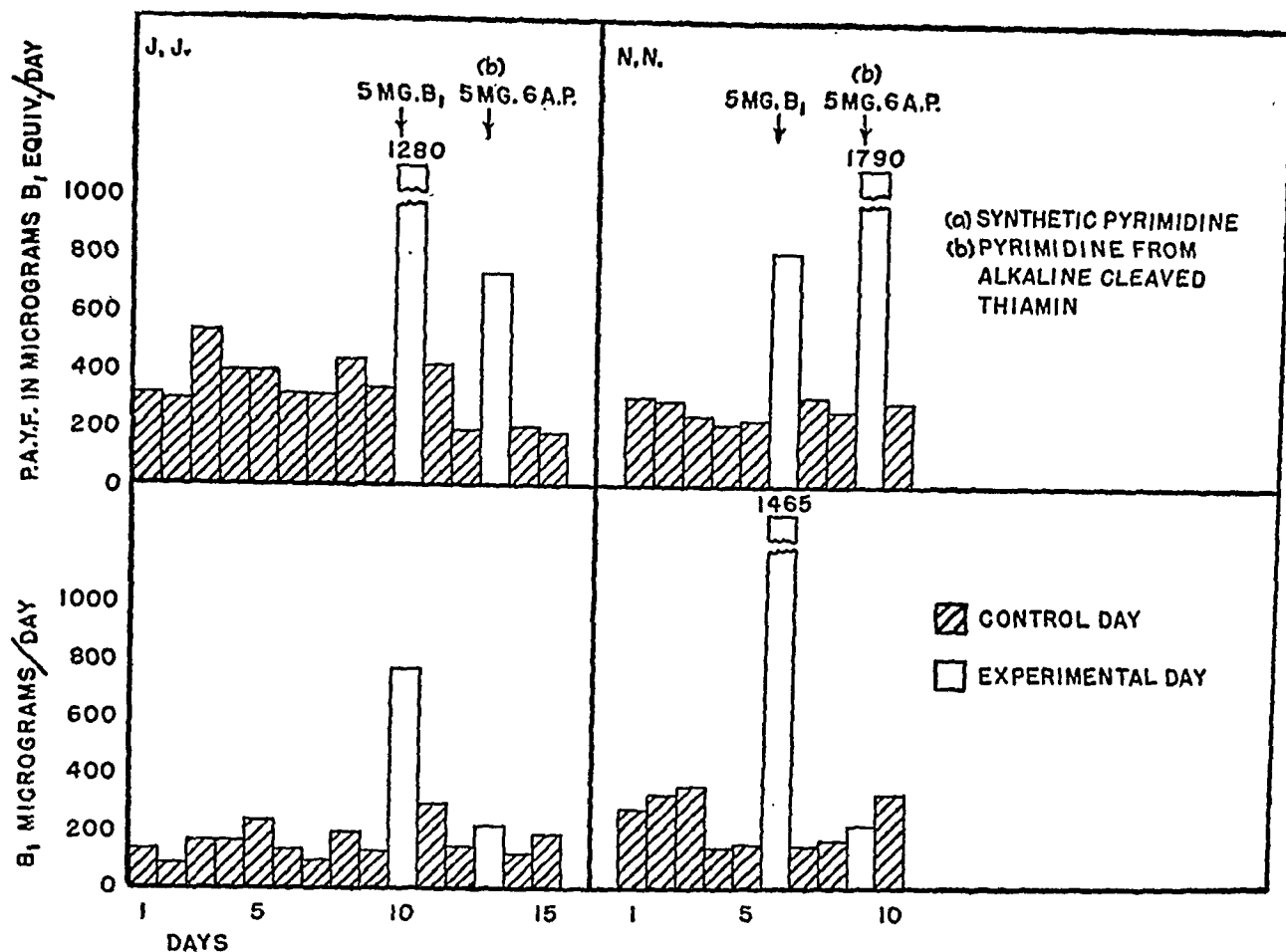


FIG. 4A

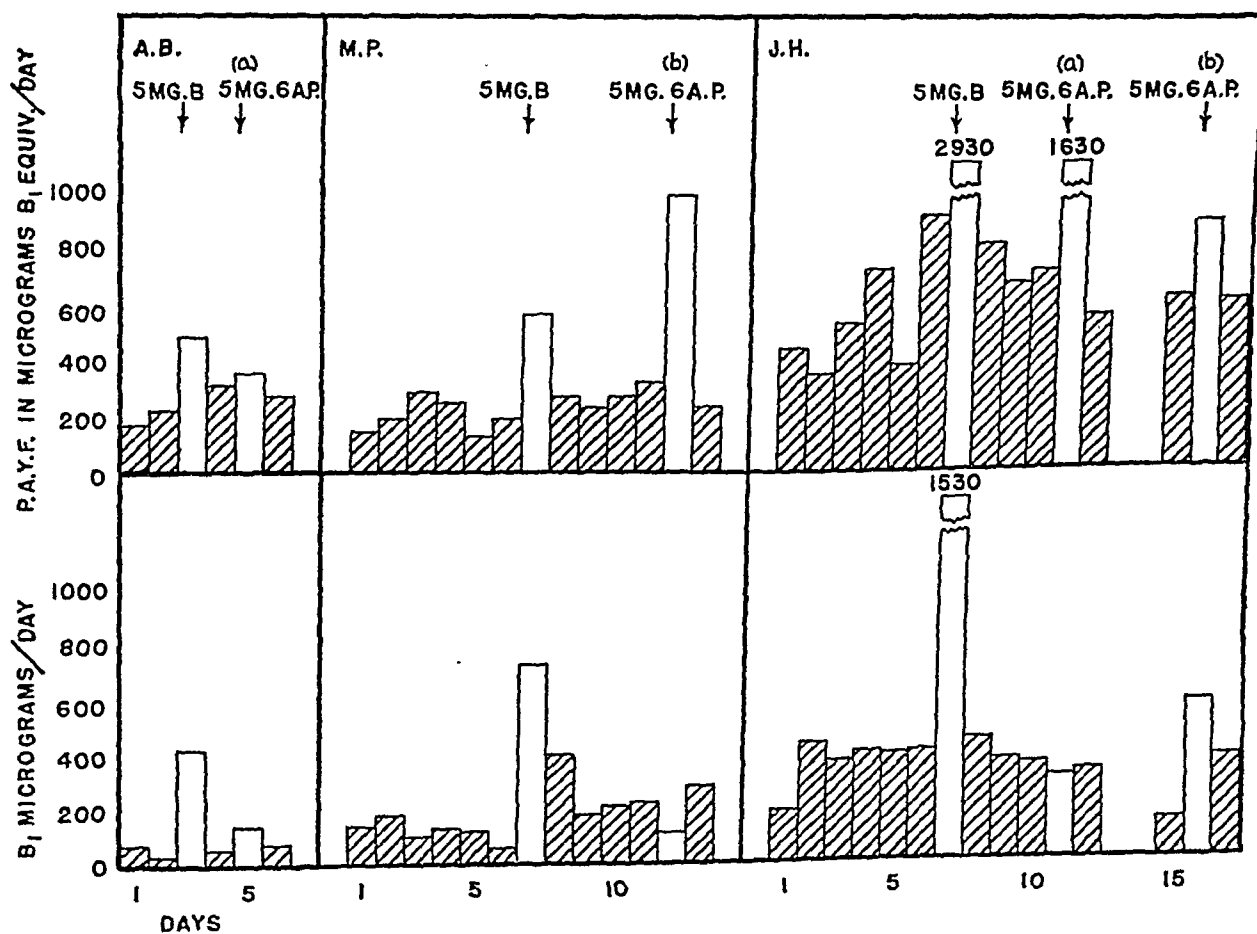


FIG. 4B

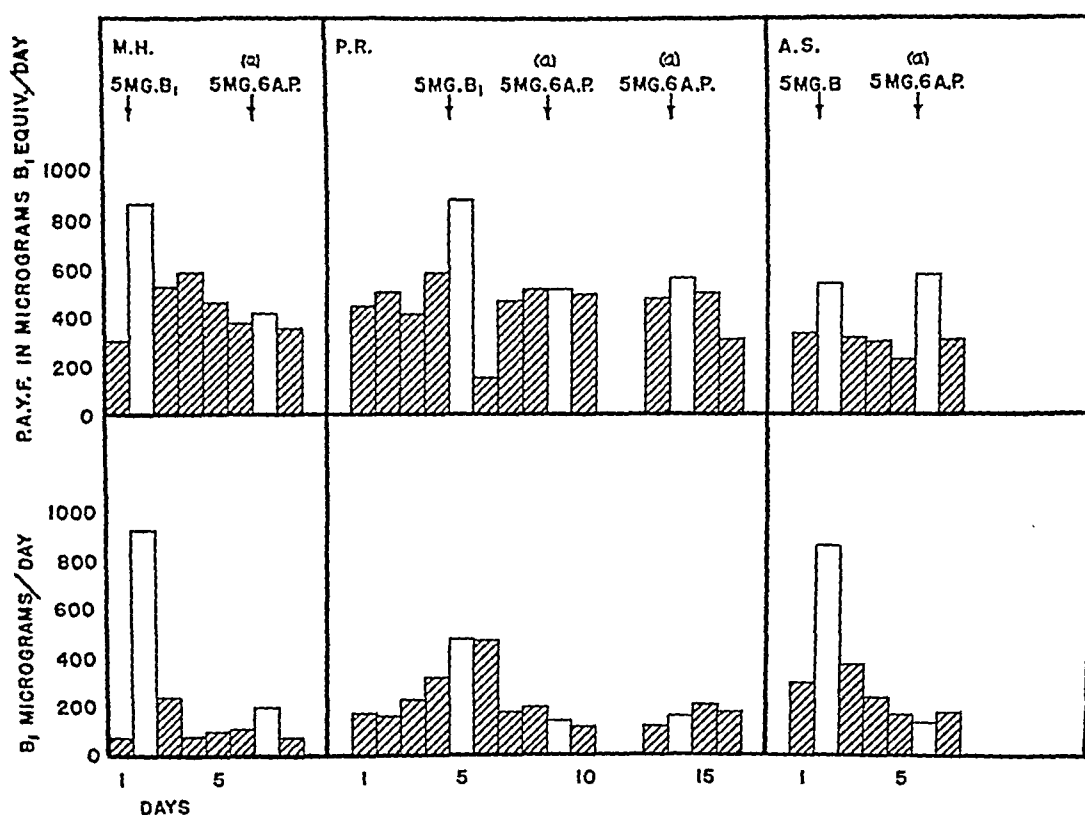


FIG. 4C

FIG. 4-A, B, C. THE URINARY EXCRETION OF THIAMIN AND OF PAYF IN NORMAL INDIVIDUALS AFTER THE INTRAVENOUS ADMINISTRATION OF 5 MGM. OF THIAMIN AND 5 MGM. OF 6-AMINO-PYRIMIDINE

travenously to 4 normal individuals. (However, the volume of this material which contained 5.0 mgm. of pyrimidine compound also contained 1.24 mgm. of thiamin.)

The results of the use of the pyrimidine formed

TABLE XV

Micrograms of thiamin excreted per day in the urine of individuals before and after the intravenous injection of 2-methyl-5-methoxyethyl-6-amino-pyrimidine

Subject	Control period				After injection		
	Number of days	Urinary thiamin range per day	Average per day	Highest per cent daily variation	24-hour out-put	Per cent increase over	
						Previous day	Average control day
M. H.	4	66-101	83	53	195	93	135
A. B.	2	25- 63	44	152	136	170	209
P. R.	4	158-314	214	99	152	-20	-28
A. S.	3	158-296	229	87	125	-20	-50
J. H.	6	179-421	350	135	558	340	60

by the cleavage of the vitamin are included in Tables XVI and XVII. The 24-hour urinary excretions of PAYF which followed the intravenous injection of the alkaline-cleavage product were significantly higher than those found after the administration of equivalent amounts of the 5-methoxy-pyrimidine, but the recoveries of PAYF in the urine still were not quantitative. The urinary excretion of PAYF by the 4 individuals during the 24 hours which followed their injection of alkaline-cleaved vitamin ranged only from 728 to 1790 micrograms, or from 100 to 589 per cent more than their average daily urinary output of the compound. A part of this increased excretion of PAYF after the administration of the cleaved thiamin might have been due to the 1240 micrograms of the vitamin which were injected simultaneously.

It is to be noted that the intravenous injection of either of these pyrimidine preparations into the 9 normal individuals was followed by a signifi-

cantly increased urinary excretion of thiamin in only 2 (Tables XV and XVII).

The results obtained in these experiments indicate that the intravenous administration of thiamin is followed consistently by significantly in-

TABLE XVI

Micrograms of PAYF excreted per day in the urine of individuals before and after the intravenous injection of the alkaline-cleaved products of thiamin*

Subject	Control period				After injection		
	Number of days	Urinary PAYF range per day	Average per day	Highest per cent daily variation	24-hour output	Per cent increase over	
						Previous day	Average control day
J. J.	9	294-525	368	178	728	287	100
J. H.	6	351-910	556	159	1630	128	193
M. P.	6	126-282	198	124	978	204	393
N. N.	5	215-300	263	40	1790	589	589

* Expressed in thiamin equivalents.

TABLE XVII

Micrograms of thiamin excreted per day in the urine of individuals before and after the intravenous injection of the alkaline-cleaved products of thiamin

Subject	Control period				After injection		
	Number of days	Urinary thiamin range per day	Average per day	Highest per cent daily variation	24-hour output	Per cent increase over	
						Previous day	Average control day
J. J.	9	76-227	140	200	202	42	44
J. H.	6	179-421	350	135	290	-15	-17
M. P.	6	53-168	111	216	102	-50	9
N. N.	5	140-348	270	143	210	31	-22

creased concentrations of PAYF in the white cells and in the urine. The injection of two biologically active pyrimidine compounds is followed by no consistent change in the urinary excretion of thiamin and by a less marked increase in urinary excretion of PAYF than that which followed the administration of thiamin. These results, then, would support the hypothesis that, normally, the PAYF which occurs in blood cells and urine is formed from the vitamin during its metabolism.

DISCUSSION

The average thiamin level of the normal white cells was found to be about 10 times that of the normal erythrocytes, a distribution similar to that of vitamin B₂ (15) and of vitamin C (16). The greater concentrations of vitamins B₁, B₂ and C in the white cells probably can be explained by the fact that, of the several blood components, the white cells most closely resemble actively metabolizing tissue. The respiratory rate (QO₂) of leukocytes and platelets also is about 100 to 1000 times that of erythrocytes (17), a ratio of a much higher order of magnitude than that of the distribution of the 3 vitamins which are known to function as essential parts of respiratory enzymes.

The present study would indicate that, in the course of its metabolism, thiamin is broken down to the PAYF compound. The evidence upon which this conclusion is based is that the intravenous administration of thiamin to normal individuals is followed consistently by a significant increase in the concentrations of the PAYF in the blood cells and urine.

It must be recognized, however, that another mechanism could explain this observation—that the PAYF in the blood and urine might have been formed not necessarily as a result of utilization of thiamin by the body, but rather by the spontaneous breakdown of the vitamin. The increased white cell content and urinary excretion of PAYF after the administration of thiamin would reflect, then, only the presence of an increased amount of thiamin with the possibility of greater total breakdown. However, if this were the case, namely, that all the PAYF was a spontaneous breakdown product of thiamin, the administration of the vitamin to all individuals under all circumstances should result in the spontaneous production of PAYF. This should be true irrespective of the existence of an abnormality in the utilization of thiamin, since such an abnormality should not alter the rate of spontaneous decomposition which the vitamin undergoes in the organism.

This explanation of spontaneous decomposition of thiamin, however, is not tenable because such an abnormality in the metabolism of the vitamin has been found in the leukemic patient. The discovery of that abnormality provided an oppor-

tunity of subjecting to experimental test the thesis just discussed. The administration of thiamin to the leukemic individual is not followed by any increase in the PAYF content of his white cells. The details of that study form the subject of a separate paper (18), but the observation is introduced here to indicate that the mere presence of thiamin is not enough to account for the simultaneous production of PAYF. The observation that an increased requirement of thiamin is associated with an increased consumption of carbohydrate foods, with pregnancy, and with fevers (19) also indicates that some of the vitamin is destroyed during its physiologic activity. At present, it would appear, therefore, that the PAYF is one of the metabolic products of thiamin in the human organism.

The work of Robbins *et al.* (14) indicates that in the animal organism thiamin cannot be synthesized from its pyrimidine analogue. Likewise, from the present experiments it would appear that in man no significant amount of the vitamin could be formed consistently from the administration either of the synthetic 2-methyl-5-methoxyethyl-6-amino-pyrimidine, or of the pyrimidine produced by the alkaline cleavage of thiamin. Both of those pyrimidines are active accelerators of yeast fermentation and are related closely in chemical structure to the pyrimidine incorporated in the thiamin molecule. However, the fact that the administration of either of those substances was followed by a smaller urinary excretion of PAYF than occurred after the administration of the vitamin itself would suggest strongly that the PAYF formed from thiamin differs from the two pyrimidines used in this investigation. Until the PAYF normally found in blood and urine can be isolated and administered, no conclusion can be drawn as to its ability to produce thiamin in man.

CONCLUSIONS

1. Methods for the determination of thiamin and the pyrimidine accelerator of yeast fermentation (PAYF) have been adapted for application to the white cells and erythrocytes of normal individuals.

2. The thiamin concentration of the leukocytes and platelets is about 10 times that of the erythro-

cytes, a distribution which probably reflects the respiratory activity of the white blood cells.

3. The white blood cell levels of thiamin reflect the thiamin deficiency and saturation of the body.

4. White cells do not differ in their capacity to absorb thiamin, but can absorb only a limited, maximum amount of the vitamin.

5. In the course of its metabolic activity, thiamin probably is broken down to the PAYF compound.

After this communication was accepted for publication, Wertz and Mitchell (20) demonstrated that the oral administration of from 2 to 4 mgm. of thiamin to normal individuals was followed by an increased urinary excretion of PAYF. These investigators conclude that PAYF is a metabolic breakdown product of the vitamin.

BIBLIOGRAPHY

1. Borson, H. J., Clinical application of the thiochrome reaction in the study of thiamin deficiency. *Ann. Int. Med.*, 1940, 14, 1.
2. Melnick, D., and Field, H., Jr., The thiamin clearance as an index of nutritional status. *J. Biol. Chem. (Proc.)*, 1941, 140, 35.
3. Harris, L. J., and Leong, P. C., The excretion of vitamin B₁ in human urine. *Lancet*, 1936, 1, 886.
4. Pollack, H., Ellenbey, M., and Dolger, H., The excretion of thiamin and its degradation products in man. *Proc. Soc. Exper. Biol. and Med.*, 1941, 47, 414.
5. Melnick, D., and Field, H., Jr., Chemical determinations of vitamin B₁. II. Method for estimation of the thiamin content of biological materials with the diazotized *p*-aminoacetophenone reagent. *J. Biol. Chem.*, 1939, 127, 515.
6. Hennessy, D. J., and Cerecido, L. R., The determination of free and phosphorylated thiamin by a modified thiochrome assay. *J. Am. Chem. Soc.*, 1939, 61, 179.
7. Atkin, L., Schultz, A. S., and Frey, C. N., Ultramicrodetermination of thiamin by the fermentation method. *J. Biol. Chem.*, 1939, 129, 471.
8. Schultz, A. S., Atkin, L., and Frey, C. N., The specificity of the fermentation test for vitamin B₁. *J. Am. Chem. Soc.*, 1938, 60, 3084.
9. Schultz, A. S., Atkin, L., and Frey, C. N., A fermentation test for vitamin B. *J. Am. Chem. Soc.*, 1937, 59, 2457.
10. Banga, I., Ochoa, S., and Peters, R. A., Pyruvate oxidation in brain. VI. The active form of vitamin B₁ and the rôle of C, dicarboxylic acids. *Bioch. J.*, 1939, 36, 1109.
11. Schultz, A. S., Light, R., and Frey, C. N., A method for the determination of thiamin and certain of its

The term "total thiamin" is used to include all these substances capable of stimulating fermentation by yeast under the conditions of the experimental procedure. The values of PAYF are expressed in micrograms of thiamin which have an equivalent yeast-stimulating activity. The true thiamin finally is calculated as the difference between total thiamin and the PAYF.

RESULTS

The results are presented in two parts: (A) The levels of thiamin and PAYF in the blood cells and urine of patients with leukemia and other diseases; and (B) Evidence by which the abnormal concentrations of these substances can be explained.

A. The levels of thiamin and PAYF in the blood cells and urine of patients with leukemia and other diseases

1. *The total thiamin concentrations in the white cells of patients with leukemia.* The concentrations of total thiamin in the white cells of 33 patients with leukemia ranged from 85 to 600 micrograms per 100 ml. of cells. The average value was 277 micrograms per 100 ml., or about 3 times the normal average (normal average is 100 micrograms). Of the 33 patients, 27 or 82 per cent, had white cell total thiamin levels above the highest normal (normal range is from 48 to 183 micrograms per 100 ml.) (Table I, Figures 1 and 2).

Of the 33 patients, 18 with myeloid leukemia had white cell thiamin concentrations which ranged from 85 to 600 micrograms per 100 ml. and averaged 296 micrograms per 100 ml. This group of patients included a child with chronic eosinophilic leukemia whose white cell thiamin value was 350 micrograms per 100 ml. of cells.

Fourteen individuals with lymphatic leukemia had white cell total thiamin levels from 160 to 520 micrograms per cent, and averaged 270 micrograms per cent. The 1 adult with chronic monocytic leukemia had a total thiamin level of 345 micrograms per 100 ml. of white cells.

The frequency with which high levels of white cell thiamin were encountered in patients with leukemia suggested immediately that the levels might reflect the type or severity of the disease or the comparative youth of the cell types affected. No such correlation could be established. Of the 33 patients, 1 with myeloid and 4 with lymphoid leukemia (Table II) were in an acute phase of the

disease marked by a predominance of blast forms. The white cells of these patients showed no greater concentrations of total thiamin than were found in the cells of patients with chronic forms of the disorder marked by more mature cellular elements.

TABLE I

Micrograms of total thiamin, true thiamin and of PAYF per 100 ml. of blood cells of patients with leukemia*

Patient	Age	Sex	Form of disease	Total thiamin		True thiamin		PAYF	
				White blood cells	Red blood cells	White blood cells	Red blood cells	White blood cells	Red blood cells
				micrograms per 100 ml.		micrograms per 100 ml.		micrograms per 100 ml.	
P.S.	19	M	Myeloid	420	20				
E.C.	18	M	Myeloid	250	19				
M.B.	26	M	Myeloid	240	20				
W.K.	48	M	Myeloid	325	30				
J.L.P.	45	M	Myeloid	235	20				
M.S.	38	M	Myeloid	85	7				
J.A.	42	M	Myeloid	410	20				
G.S.	52	M	Myeloid	240	6				
N.M.	12	F	Myeloid	350	25				
S.M.C.	60	F	Myeloid	150	14				
A.F.	53	F	Myeloid	275	14				
M.B.	13	F	Myeloid	300	12				
I.L.	26	F	Myeloid	565	68				
E.S.	4	M	Lymphoid	240	14				
S.G.	38	M	Lymphoid	170	22				
L.G.	10	M	Lymphoid	520	69				
D.P.	51	M	Lymphoid	300	18				
J.V.	52	M	Lymphoid	180	18				
M.V.	54	M	Lymphoid	340	15				
J.R.	47	M	Lymphoid	160	17				
J.T.	4	M	Lymphoid	165	25				
S.H.	61	M	Lymphoid	280	24				
T.M.	7	M	Lymphoid	225	16				
J.K.	7	M	Lymphoid	170	6				
P.K.	6	F	Lymphoid	430	9				
J.B.	50	M	Monocytic	534	32				
S.G.	35	F	Myeloid	302	25	266	17.7	36	7.3
M.K.	40	F	Myeloid	232	9.4	221	9.4	11	1.0
J.K.	37	F	Myeloid	200	20.5	167	18.0	33	2.5
L.J.	50	M	Myeloid	230	9	226	7.4	4	1.6
J.W.	55	M	Myeloid	600	41	569	20.4	31	21.6
A.M.	48	M	Lymphoid	256	12.9	246	11.9	10	1.0
A.B.	54	M	Lymphoid	238	36.6	214	28.6	24	8.0
			Average	277	22	273	26.2	21.3	6.1

* Expressed in thiamin equivalents.

The white cell thiamin of the group with acute leukemia varied from 220 to 425 micrograms per 100 ml. of cells and averaged 277 micrograms per 100 ml., whereas that of the remainder of the patients with chronic leukemia ranged from 83 to 600 micrograms per 100 ml., and averaged 272 micrograms per 100 ml.

Three patients with lymphoid leukemia were in an aleukemic phase. Although the disorder in these patients was marked by relatively few apparently immature circulating leukocytes (Table III),

these 3 also had high white cell total thiamin levels: 165, 240 and 280 micrograms per cent.

2. *The PAYF concentrations in the white cells of patients with leukemia.* The levels of PAYF

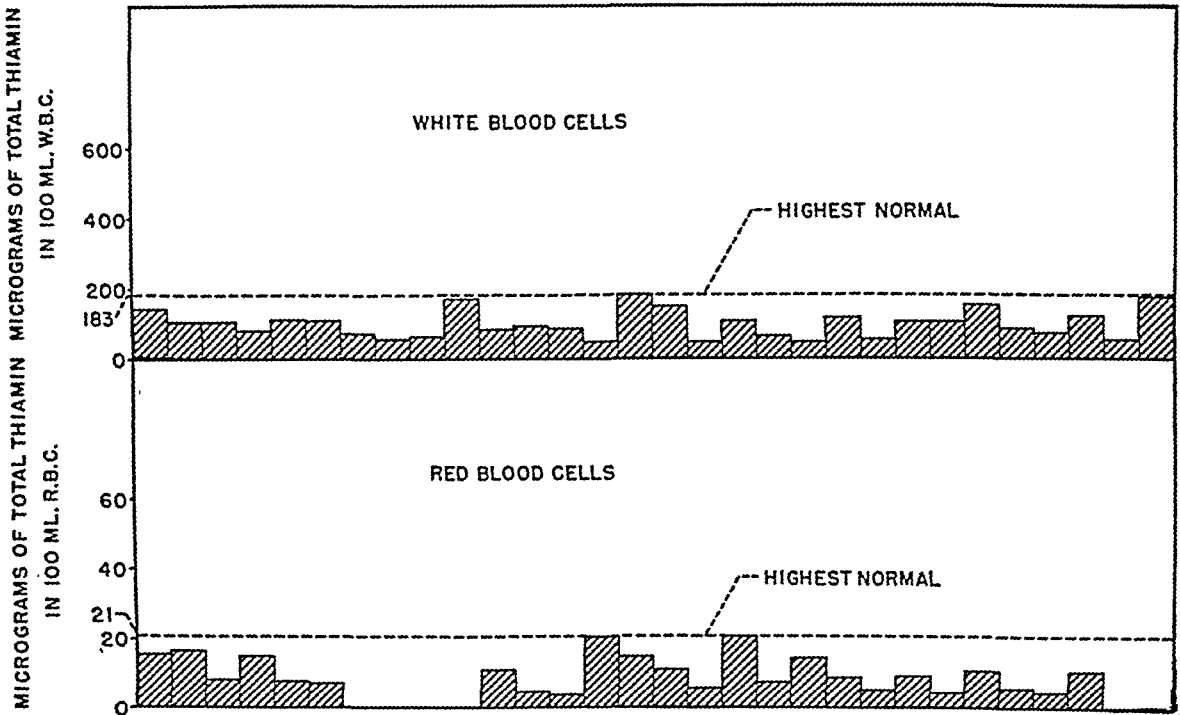


FIG. 1. THE LEVELS OF TOTAL THIAMIN IN THE BLOOD CELLS OF NORMAL INDIVIDUALS

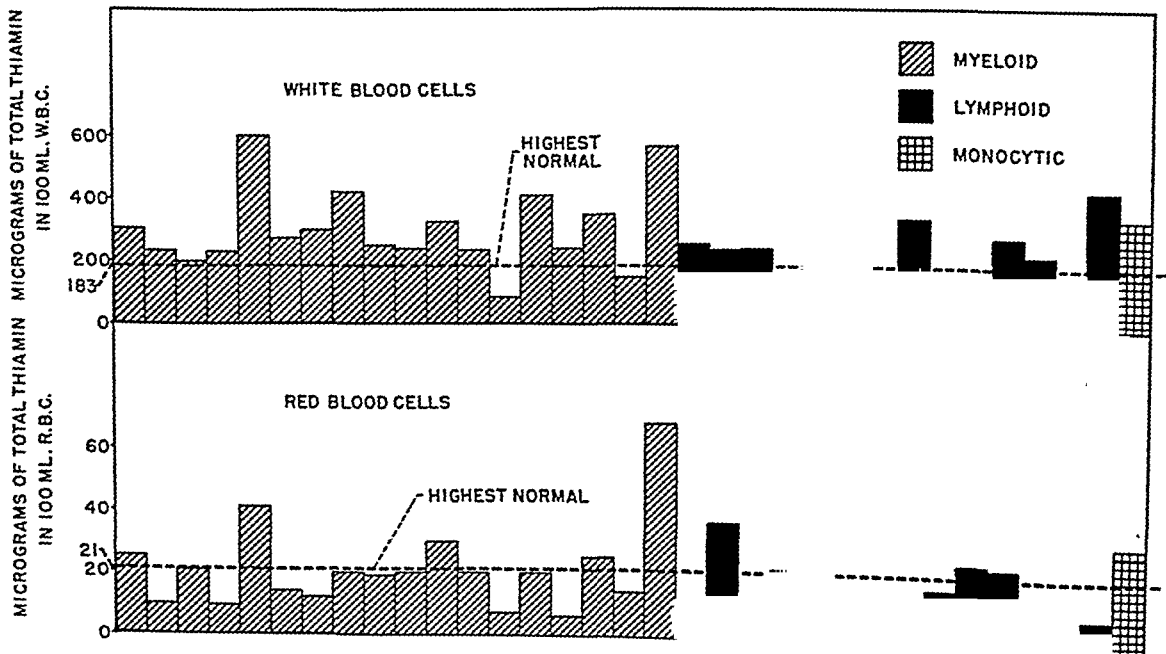


FIG. 2. THE LEVELS OF TOTAL THIAMIN IN THE BLOOD CELLS OF PATIENTS WITH LEUKEMIA

TABLE II

The blood cell content of total thiamin of patients with acute leukemia

Patient	Form	Blood count	Marrow	Total thiamin	
				White blood cells	Red blood cells
E. C.	Myeloid	Red blood cells 2.21, Hemoglobin 42% White blood cells 31,600 Myeloblasts 60% Myelocytes 15% Polymorphonuclear leukocytes 18% Lymphocytes 7%	Myeloblasts 85%	<i>micrograms per 100 ml.</i> 250	20
E. S.	Lymphoid	Red blood cells 1.50, Hemoglobin 32% White blood cells 2800 Lymphoblasts 80% Polymorphonuclear leukocytes 20%	Lymphoblasts 100%	240	14
P. C.	Lymphoid	Red blood cells 3.75, Hemoglobin 40% White blood cells 57,200 Lymphoblasts 90% Polymorphonuclear leukocytes 5% Monocytes 5%	Lymphoblasts 100%	425	9
A. B.	Lymphoid	Red blood cells 2.44, Hemoglobin 45% White blood cells 160,000 Lymphoblasts 100%	Lymphoblasts 100%	220	30
E. D.	Lymphoid	Red blood cells 3.04, Hemoglobin 61% White blood cells 31,000 Lymphoblasts 98% Polymorphonuclear leukocytes 2%	Lymphoblasts 100%	250	14
Average				277	17.6

TABLE III

Total thiamin in the blood cells of patients with aleukemic leukemia

Patient	Form	Blood	Marrow	Total thiamin	
				White blood cells	Red blood cells
S. H.	Chronic lymphoid	Red blood cells 1.76, Hemoglobin 39% White blood cells 4600 Lymphocytes 81% Polymorphonuclear leukocytes 12% Monocytes 7%	Lymphoblasts 10% Lymphocytes 60%	<i>micrograms per 100 ml.</i> 280	24
J. T.	Chronic lymphoid	Red blood cells 3.42, Hemoglobin 57% White blood cells 5800 Lymphocytes 68% Polymorphonuclear leukocytes 26% Monocytes 6%	Lymphoblasts 16% Lymphocytes 58%	165	25
C. S.	Chronic lymphoid	Red blood cells 3.23, Hemoglobin 72% White blood cells 5500 Polymorphonuclear leukocytes 63% Metamyelocytes 5% Monocytes 8% Lymphocytes 8%	Myeloblasts 4% Myelocytes 40%	240	12
Average				228	27.3

were determined in the leukocytes and platelets of 7 of the 34 patients with all forms of leukemia (Table I). These levels ranged from 4 to 36 micrograms and averaged 21 micrograms per 100 ml. It is interesting that, whereas the PAYF formed from 16.8 per cent to 64.0 per cent (average 30.5 per cent) of the total thiamin of *normal* white cells, in the *leukemic* white cells that substance represented only from 2.0 to 16.5 per cent (average 7.7 per cent) of the total thiamin. It should be noted that no leukemic patient showed a per cent PAYF of the total thiamin as high as that of the lowest normal (16.8 per cent).

3. *The total thiamin concentration in the erythrocytes of patients with leukemia.* The total thiamin levels in the red cells were determined in all of the 33 patients with leukemia (Table I). In general, these levels were higher than the concentrations of total thiamin in normal red cells (1), but were not as abnormally elevated as were the levels in the leukemic white cells. The total thiamin concentrations in the erythrocytes of the patients with leukemia varied from 6 to 69 micrograms and averaged 22 micrograms per 100 ml., or about twice the normal value (normal average 10.0 micrograms). Of the 33 patients, 11, or 32 per cent, had erythrocyte total thiamin levels above the highest normal value, and 28, or 82 per cent, had levels above the normal average. The frequency with which elevated erythrocyte thiamin levels were encountered is further evidence against the view that the abnormally high levels in leukemic leukocytes reflect simply the immaturity of the cells.

No apparent correlation has been found between the erythrocyte level of total thiamin and the form or severity of the leukemic process.

In the 18 patients with myeloid leukemia whose erythrocyte thiamin levels were determined, those levels varied from 6 to 68 micrograms and averaged 21 micrograms per cent. Similarly, in the 14 patients with lymphoid leukemia, the erythrocyte levels of total thiamin ranged from 6 to 69 micrograms and averaged 23 micrograms per cent.

The thiamin levels in erythrocytes of the 5 patients with acute leukemia ranged from 9 to 30 micrograms and averaged 18 micrograms per 100 ml., a value only a little less than that of all the patients studied (21 micrograms). Finally, the 3

patients whose disease was in the aleukemic state also had elevated levels of the vitamin in their red cells: 12, 24 and 25 micrograms per 100 ml.

4. *The concentrations of PAYF in the erythrocytes of patients with leukemia.* The concentrations of PAYF were determined in the red cells of 7 of the 33 patients (Table I). These concentrations ranged from 1 to 22 micrograms and averaged 6 micrograms per cent. The PAYF thus formed from 8 per cent to 51 per cent of the total thiamin of the red cells, values which are apparently within the normal range (14.0 to 30.0 per cent).

Since the blood corpuscles of leukemic individuals contain excessive amounts of total thiamin, the results here presented would explain and confirm the observations of Sinclair (2) and of Goodhart and Sinclair (3) that leukemic patients occasionally have high concentrations of thiamin and cocarboxylase in their whole blood. It would appear that had proper corrections been made for the anemia of the blood specimens which those investigators examined, a higher incidence of abnormally high thiamin and cocarboxylase levels would have been found.

TABLE IV

Micrograms of thiamin and of PAYF excreted per day in the urine of patients with leukemia*

Subject	Weight	Number of days	Urinary thiamin range per day	Average per day	Highest per cent daily variation	Urinary PAYF range per day	Average per day	Highest per cent daily variation
	<i>kilos</i>							
J.K.	70	5	238-403	327	69	272-419	360	54
P.S.	68	10	57- 74	65	23	192-243	209	26
M.L.	60	2	70- 83	76	9	40- 46	43	8
L.J.	85	4	44- 90	78	110	55-135	115	145
J.W.	72	3	65-215	165	230	245-301	256	231

* Expressed in thiamin equivalents.

5. *The urinary excretion of total thiamin and PAYF by patients with leukemia.* The excretions of thiamin and of PAYF have been determined in thirteen urine specimens of 5 adults with leukemia (Table IV). The urine collections were made over periods of from 1 to 4 days, and all of the patients had been on an adequate hospital diet without added thiamin for several days before the collections were begun.

The excretion of true thiamin by these 5 patients ranged from 44 to 403 micrograms per day. The average daily true thiamin output of each over periods of from 2 to 10 days varied between 65 and 327 micrograms and averaged 142 micrograms. These values are similar to those of the average urinary excretion of true thiamin by each of 8 *normal* individuals which ranged from 83 to 350 micrograms and averaged 180 micrograms per day (1).

The excretion of PAYF in the thirteen urine specimens of the leukemic patients ranged from 40 to 419 micrograms per day, and the average daily output of each ranged from 43 to 360 micrograms. Similarly, the average urinary output of PAYF of each of 8 *normal* individuals varied from 194 to 556 micrograms per day, and averaged 340 micrograms per day (1). Thus the values for the urinary excretion of true thiamin and PAYF by the 5 leukemic patients were all within normal limits.

In summary, it has been found that the total thiamin levels in the white cells of 33 patients with various types of leukemia average about 3 times the normal average value, and in 85 per cent of the patients are above the highest normal. The PAYF concentration forms a smaller percentage of the total thiamin in the leukemic white cells of all patients than it does in the white cells of any normal individual studied. The average concentration of total thiamin in the erythrocytes of 33 leukemic patients also is abnormally high—about twice the normal average concentration—but only 32 per cent of the patients studied had red cell total thiamin levels above the highest normal. In contrast to the finding in white cells, the PAYF in the erythrocytes of leukemic patients forms a normal percentage of the total thiamin. Finally, the patients with leukemia excrete normal amounts of thiamin and PAYF.

6. *The concentration of total thiamin in the blood cells of patients with malignant diseases other than leukemia.* It was important to determine whether or not high total thiamin concentrations were unique for the blood cells of leukemic patients. Therefore, the blood cells of groups of patients with other diseases of equal gravity were studied.

Of 8 patients with Hodgkin's disease (Table V), 6 had white cell concentrations of total thia-

TABLE V
Blood cell total thiamin in patients with Hodgkin's disease

Patient	Total thiamin	
	White blood cells	Red blood cells
	<i>micrograms per 100 ml.</i>	
R. E.....	420	10.2
A. G.....	160	24.0
M. K.....	329	19.2
P. K.....	274	16.4
R. N.....	264	18.0
L. R.....	120	13.1
J. R.....	193	10.0
F. G.....	187	8.6
Average.....	242	14.0

min above the normal range. The values varied from 120 to 420 micrograms and averaged 242 micrograms per 100 ml. of cells, or 2.5 times the normal average value. The range of the total thiamin in the red cells of this group of patients was from 8.6 to 24.0 micrograms and averaged 14.0 micrograms per 100 ml., or about 1.4 times the normal average level. It is interesting to note that this high concentration of thiamin in the blood cells of patients with Hodgkin's disease is the first indication that these cells are in any way abnormal. Furthermore, this observation suggests that a similar metabolic abnormality marks a relationship between the blood cells of patients with Hodgkin's disease and those of patients with leukemia.

Of 24 patients with cancer of the gastro-intestinal tract, the white cell concentrations of total thiamin ranged from 33 to 400 micrograms per cent, and the average was 179 micrograms per cent, or about twice the normal average value (Table VI). Of the 24 patients, 11, or 46 per cent, had white cell thiamin levels above the highest normal value. The erythrocyte thiamin levels of 15 of these 24 patients also were determined. These ranged from 4 to 24 micrograms and averaged 14 micrograms per 100 ml. Only 1 of the 15 individuals had an erythrocyte thiamin level above the normal range.

Of 5 patients with cirrhosis of the liver (Table VII), the white cell concentrations of total thiamin in 3 were within normal limits, and in the other 2 somewhat below the normal range. These concentrations varied from 27 to 175 micrograms and averaged 98 micrograms per 100 ml. The red cell total thiamin was determined in 4 of these 5

TABLE VI
Blood cell total thiamin in patients with cancer
of the gastro-intestinal tract

Patient	Total thiamin	
	White blood cells	Red blood cells
	micrograms per 100 ml.	
R. M.	164	11
L. B.	231	16
K. B.	128	13
E. B.	115	18
F. F.	284	17
F. M.	226	24
R. K.	321	18
M. M.	400	16
I. C.	58	
M. P.	51	12
J. P.	292	19
F. P.	274	13
J. S.	33	
O. S.	214	4
F. W.	110	8
R. L.	202	13
A. S.	168	6
O. J.	100	
S. K.	80	
L. L.	210	
G. B.	236	
J. W.	131	
F. C.	115	
S. T.	141	
Average	179	14

patients and ranged from 1.7 to 16.5 micrograms per cent and averaged 7.6 micrograms per cent.

It would appear, therefore, that elevated blood cell concentrations of total thiamin are not specific for patients with leukemia, but are to be found also in the morphologically normal blood cells of patients with other neoplastic growths. This fact also indicates that the high thiamin levels in leukemic leukocytes and platelets are not due to the immaturity of the cells concerned.

TABLE VII
Blood cell total thiamin in patients with cirrhosis of liver

Patient	Total thiamin	
	White blood cells	Red blood cells
	micrograms per 100 ml.	
M. R.	27	1.7
H. R.	153	16.5
M. S.	94	7.1
I. T.	175	5.2
H. H.	35	

B. Experiments to determine the cause of the high concentrations of thiamin in the blood cells of patients with leukemia

The most probable explanations of the high concentrations of total thiamin in the blood cells

of patients with leukemia are that the patients had (1) a high dietary intake of thiamin, (2) white cells, which because of their youth, have an accelerated rate of normal metabolism, (3) impaired utilization of the vitamin, or (4) faulty excretion of the vitamin.

1. *Were the high thiamin levels of leukemic white cells due to excessive ingestion of thiamin?* Since it has been demonstrated in the previous communication (1) that the daily intake of from 10 mgm. to 100 mgm. of thiamin for from 3 to 7 days can elevate the white cell content of the vitamin to the range found in leukemic leukocytes and platelets, it was necessary to determine whether or not the leukemic patients had ingested excessive amounts of thiamin. When the dietary histories of the patients were examined, none indicated that any supplements of thiamin or of thiamin-rich extracts had been taken for at least 3 weeks before admission to the hospital. It is therefore impossible to attribute the occurrence of high concentrations of blood cell thiamin to an abnormally high intake of the vitamin.

2. *Were the high thiamin levels of leukemic white cells a function of their youth and consequently accelerated rate of normal metabolism?* The frequent occurrence of high levels of thiamin in leukemic white cells suggested that such elevations were due to the comparative youth of the cell types affected. From observations already presented this explanation appeared to be untenable. It has been pointed out that:

a. No significant difference was found between the concentrations of total thiamin in the white cells of patients with acute leukemia and of patients with aleukemic leukemia, although the white cells of the latter group were much more mature than those of the former.

b. The concentrations of total thiamin were elevated in the white cells of 75 per cent of patients with Hodgkin's disease, and of 46 per cent of patients with cancer of the gastro-intestinal tract. These white cells apparently were morphologically mature.

c. Abnormally elevated concentrations of total thiamin were found in the red cells of the patients with leukemia. There was no reason to believe that those cells were any younger than the erythrocytes normally found in healthy individuals.

Despite this evidence it was desirable to study further the possibility that the thiamin concentration of cells was a function of their age and development, and that higher concentrations existed in the younger cells. If it could be demonstrated, however, that the thiamin levels in normal myelocytes or erythroblasts were not significantly higher than those in the mature white cells of the peripheral blood, such a hypothesis could be ruled out.

Accordingly, two suspensions of rabbit myelocytes and two of erythroblasts were made by the methods of Warren (5) from the white and red marrow, respectively. Three suspensions of mature white cells from whole blood were prepared by the techniques already described. Determinations of the total thiamin levels of all of these suspensions revealed no significant differences, and all the levels were within the range of those found in normal mature white cells (Table VIII).

TABLE VIII

The total thiamin in the marrow myelocytes, marrow erythroblasts, peripheral white cells, and peritoneal white cells of rabbits

	Total thiamin			
	Peripheral white cells	Marrow myelocytes	Marrow erythroblasts	Peritoneal white cells
	<i>micrograms per 100 ml.</i>			
Rabbit number 1	100	171		328 300
Rabbit number 2	80	99		307 206
Rabbit number 3	137		87.5	
Rabbit number 4			140	

The total thiamin concentrations of the three preparations of mature white cells from rabbit blood were 80, 100, and 137 micrograms per cent; those of the erythroblasts 88 and 140 micrograms per cent, and those of the myeloblasts 99 and 171 micrograms per cent. Since the immature nucleated marrow cells did not have the abnormally high concentrations of the vitamin such as were found in leukemic white cells, the possibility was excluded that the high thiamin content of leukemic leukocytes and platelets reflected only their comparative youth.

A second approach to this problem was attempted. By the repeated injection of saline into the peritoneal cavity of a rabbit, and the withdrawal of the fluid 8 hours later, it is possible to recover progressively younger leukocytes in each successive exudate (6). In this manner two suspensions of polymorphonuclear cells and one of metamyelocytes were obtained for a comparison of their concentrations of intracellular thiamin. The measurement of the vitamin in these suspensions thus would reveal whether or not higher concentrations of thiamin existed in younger cells.

It was found, however, that the two suspensions of adult polymorphonuclear cells had thiamin levels of 328 and 307 micrograms per 100 ml., whereas the subsequently formed metamyelocytes had a thiamin concentration of 300 and 206 micrograms per 100 ml. Therefore, it appears that not only do the younger metamyelocytes have less thiamin than do the adult polynuclears, but both cell types obtained from the exudates have concentrations of the vitamin considerably higher than those in the adult white cells of the peripheral blood, or in the immature marrow cells. It is possible that this discrepancy might be due to the fact that the exudate cells, by the nature of their formation, are injured cells, and thus are not comparable to peripheral or marrow blood cells.

From the evidence at hand, however, it would appear that the concentration of thiamin in the white cells is not a function of the age or development of those cells. Therefore, some other explanation must be sought to explain the high levels of thiamin in leukemic leukocytes.

3. *Were the high blood cell thiamin levels due to an impaired utilization of the vitamin by the cells?* In the communication previously referred to (1), a method was described for the study of the relationship between thiamin and PAYF in the normal blood cells and urine. It appeared from the evidence that thiamin, during its normal physiologic activity, probably is converted in the normal white cells to the PAYF compound. This conclusion was based on the observation that the intravenous administration of thiamin to normal individuals was followed consistently by an immediate and significant increase in the concentrations of thiamin and PAYF in both the white cells and urine.

In section A. 1. of this study, it was shown that not only were the concentrations of blood cell total thiamin abnormally high in the leukemic patients, but also that the PAYF content of the leukocytes and platelets formed a much smaller percentage of the blood cell content of total thiamin than it did in normal white cells. This discrepancy suggested, therefore, that in the leukemic white blood cells the rate or degree of formation of PAYF from the vitamin was decreased.

In order to test that possibility further, it was necessary to study in leukemics the effects of the intravenous administration of thiamin and its probable normal metabolite, the PAYF compound, on the contents of those substances in the blood and urine. Six patients with chronic leukemia—3 females and 3 males, 5 with myeloid and 1 with lymphoid disease—were used for this study. The experiments were done under the same conditions, and in the same manner as described in the preceding paper (1). Five of the 6 patients received both the thiamin and, several days later, 2-

methyl-5-methoxyethyl-6-amino-pyrimidine. One patient received only the intravenous injection of the thiamin.

The results obtained after the administration of the test substances to the leukemic individuals were in sharp contrast to those observed in normal controls. The intravenous administration of 5 mgm. of thiamin to each of the 5 normal adults had been followed by a *rise* within the first 3 hours of the white cell thiamin from 80 per cent to 132 per cent, and the average rise was 100 per cent. Likewise, there followed a *rise* in the white cell PAYF of each individual from 41 per cent to 173 per cent, and the average rise was 143 per cent. In the 6 leukemic patients, on the other hand, the administration of 5 mgm. of thiamin intravenously was followed in every instance by a *fall* in the white cell concentrations of thiamin, and in 4 of the 6 by a *fall* in the PAYF as well (Table IX) (Figure 3). The thiamin concentration decreased within the first 3 hours after the injection by from 4 per cent to 59 per cent, and the average

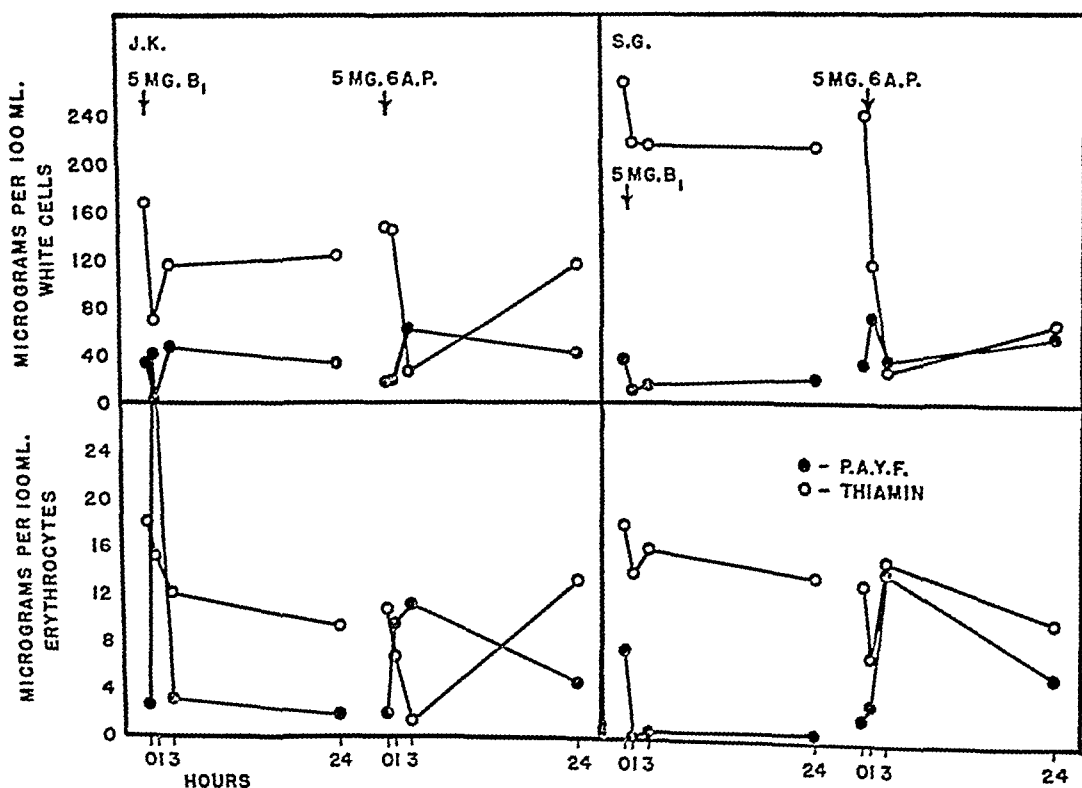


FIG. 3. THE LEVELS OF THIAMIN AND OF PAYF IN WHITE CELLS AND ERYTHROCYTES OF PATIENTS WITH LEUKEMIA AFTER THE INTRAVENOUS ADMINISTRATION OF 5 MGm. OF THIAMIN AND 5 MGm. OF 2-METHYL-5-METHOXYETHYL-6-AMINO PYRIMIDINE

decrease was 32 per cent. The PAYF of the leukemic white cells of the 4 patients decreased by from 36 per cent to 72 per cent in the first 3 hours, and the average decrease was 60 per cent.

TABLE IX

The concentrations of true thiamin and of PAYF in the white cells and erythrocytes of patients with leukemia given thiamin and 2-methyl-5-methoxyethyl-6-amino-pyrimidine*

Patient	Given	Hours after injection	White blood cells		Red blood cells	
			True thiamin	PAYF	True thiamin	PAYF
			<i>micrograms per 100 ml.</i>		<i>micrograms per 100 ml.</i>	
S. G.	5 mgm. B ₁	0	266	36	17.7	7.3
		1	216	10	13.7	0
		3	213	15	15.8	0.6
		24	212	16	13.6	0.5
	5 mgm. 6AP	0	238	33	13	1.9
		1	115	70	7	3.1
		3	27	36	15	14
		24	66	56	10	5.5
M. K.	5 mgm. B ₁	0	221	10.7	9.4	0
		1	212	11.2	10.4	2.0
		3	223	6.9	9.7	2.1
		24	229	10.9	6.5	1.2
	5 mgm. 6AP	0	183	23.7	8.2	1.7
		1	171	21.6	9.3	8.7
		3	218	43.0	10.1	4.4
		24	211	11.0	6.1	0.5
J. K.	5 mgm. B ₁	0	167	33	18	2.5
		1	69	2.4	15.2	32.0
		3	116	47.5	12.0	3.1
		24	123	33.5	9.2	1.7
	5 mgm. 6AP	0	146	17.5	10.6	1.8
		1	144	18.3	6.1	9.5
		3	26.3	61.7	13.1	4.5
		24	116	40.4		
L. J.	5 mgm. B ₁	0	226	3.8	7.4	1.6
		1	142	9.0	14.4	1.6
		3	113	41.0	3.9	6.1
		24	320	20.0	8.0	13.0
	5 mgm. 6AP	0	371	20	7	14
		1	253	104	35	1
		3	201	79	17.4	2.6
		24	94	143	10.2	8.0
A. M.	5 mgm. B ₁	0	246	10	11.9	1.0
		1	157	12.9	9.9	0.9
		3	222	4.4	3.3	7.4
		24	285	21.4	11.8	2.1
J. W.	5 mgm. B ₁	0	493	10	40	1.0
		1	353	9.4	18	5.0
		3	423	11.5	18	4.0
		24	462	61	11	20.0
	5 mgm. 6AP	0	569	31	20.4	21.6
		1	600	65.4	18	22
		3	369	84.0	18	19
		24	634	11.0	6	34

In the other 2 of the 6 patients, the administration of thiamin was followed by a rise in the white cell content of PAYF of from 4 to 41 micrograms per cent (L. W.) and from 10 to 60 micrograms per cent (J. W.).

Although the administration of 5 mgm.³ of 2-methyl-5-methoxyethyl-6-amino-pyrimidine to 5 normal individuals was followed in every instance by a *rise* in the white cell content of PAYF, it was not followed by any rise in the white cell thiamin in 3 of the 5 individuals. In the white cells of 5 leukemic patients to whom 5 mgm. of 2-methyl-5-methoxyethyl-6-amino-pyrimidine were administered intravenously, the content of PAYF rose in all by from 100 per cent to 420 per cent. The average rise was 249 per cent. It is interesting to note, however, that the injection was followed consistently within the first 3 hours by a *fall* in white cell thiamin. This fall ranged from 6.5 per cent to 82.0 per cent.

Thus it seems that some deviation from the normal handling of administered thiamin exists in the leukemic leukocytes and platelets. The evidence which indicates such an aberration is as follows: (1) Under fasting conditions PAYF, a probable normal metabolite of thiamin, formed an abnormally small percentage of the total thiamin content. (2) The intravenous administration of the thiamin to leukemic patients was always followed by a *fall*, and not a *rise*, in the white cell concentration of thiamin. (3) The administration of the thiamin was followed by a *fall*, and not a *rise*, in the PAYF content in the white cells of 4 of 6 of the patients. In the leukemic, as in the normal, white blood cells there is no reason to believe that thiamin is formed from administered pyrimidine.

Similar measurements were made in the erythrocytes of the patients with leukemia who had received thiamin and 2-methyl-5-methoxyethyl-6-amino-pyrimidine. As in the previous study of normal individuals, no consistent results were obtained. Only the results of the administration of the test substances to the leukemic patients are shown in Table IX and Figure 3 and no attempt has been made to draw any conclusions from them.

The existence of an abnormal metabolism of thiamin in the patient with leukemia is supported to some degree by the results of experiments in

³ Equivalent to 10.7 mgm. of thiamin in yeast-stimulating activity.

which measurements of the urinary concentrations of thiamin and pyrimidine were made after the injection of those substances. These experiments, too, were performed exactly as described in the previous communication (1).

In the 8 normal individuals used for the previous study, it was demonstrated that the intravenous administration of 5 mgm. of thiamin in every instance was followed during the next 24 hours by a significant increase in the urinary excretion of both thiamin (from 121 to 835 per cent of the average control level) and PAYF (from 89 to 428 per cent of the average control level). On the other hand, no significantly increased excretion of thiamin occurred during the administration of either 2-methyl-5-methoxyethyl-6-aminopyrimidine or the pyrimidine formed by the alkaline cleavage of the vitamin itself. These observations thus lent further support to the hypothesis that in normal individuals thiamin is converted to the pyrimidine compound.

TABLE X

Micrograms of thiamin excreted per day in the urine of patients with leukemia before and after the intravenous injection of thiamin

Patient	Number of days	Control period			After injection		
		Urinary thiamin range per day	Average per day	Highest per cent daily variation	24-hour output	Per cent increase over	
						Previous day	Average control day
J. K.	5	238-403	327	69	1263	430	278
M. K.	2	70- 83	76	9	722	720	850
L. J.	4	44- 90	78	110	1146	3200	950
J. W.	3	65-215	165	230	326	310	98

In sharp contrast to these findings are the results of a similar study of leukemic individuals. Five mgm. of thiamin were administered to each of 4 patients with chronic leukemia—2 females and 2 males, 3 with myeloid and 1 with lymphoid disease. In all 4, the administration of the thiamin increased the urinary excretion of the vitamin during the next 24-hour period by from 30 per cent to 3200 per cent (Table X). These increases are significantly higher than those which the injection of the same amount of thiamin affected in the normal controls (121 to 835 per cent).

The leukemic patients excreted only a little less pyrimidine after the injection of thiamin than did the normals. In all 8 normals the injection of 5 mgm. of the vitamin had resulted in increased urinary output of PAYF which ranged from 89 per

TABLE XI

Micrograms of PAYF excreted per day in the urine of patients with leukemia before and after the intravenous injection of thiamin*

Patient	Number of days	Control period			After injection		
		Urinary PAYF range per day	Average per day	Highest per cent daily variation	24-hour output	Per cent increase over	
						Previous day	Average control day
J. K.	5	272-419	360	54	448	70	24.5
M. K.	2	40- 46	43	8	214	366	394
L. J.	4	55-135	115	145	199	261	73
J. W.	3	245-301	256	231	519	71.5	104

* Expressed in thiamin equivalents.

cent to 428 per cent, and averaged 189 per cent above their average output. In the 4 leukemic patients, the changes in the urinary excretion of that substance during the succeeding day ranged from 24 to 394 per cent above the average output, and averaged 143 per cent (Table XI).

The 4 patients also received intravenously 5 mgm.⁴ of 2-methyl-5-methoxyethyl-6-aminopyrimidine. The results of this experiment were, in general, the same as those in which the compound was administered to normal individuals. The injection produced during the next 24 hours an increased output of thiamin (128 per cent) in only 1 patient, and an increased output of PAYF (by from 102 to 1105 per cent) in all (Tables XII and XIII).

From these measurements of urinary thiamin and PAYF it would appear that the leukemic patient utilized less of the administered vitamin than did the normal individual.

4. *Were the high blood cell thiamin levels due to faulty excretion of the vitamin?* It is obvious that should any interference in the excretion of thiamin exist in patients with leukemia, a cause for the high levels of the vitamin in the blood

⁴ Equivalent to 10.7 mgm. of thiamin in yeast-stimulating activity.

TABLE XII

Micrograms of PAYF excreted per day in the urine of patients with leukemia before and after the intravenous injection of 2-methyl-5-methoxyethyl-6-amino-pyrimidine*

Patient	Number of days	Control period			After injection		
		Urinary PAYF range per day	Average per day	Highest per cent daily variation	24-hour output	Per cent increase over	
						Pre-vious day	Average control day
J. K.	5	272-419	360	54	730	154	102
M. K.	2	40- 46	43	8	536	1006	1105
L. J.	4	55-135	115	145	327	23	185
J. W.	3	245-301	256	231	1152	368	350

* Expressed in thiamin equivalents.

TABLE XIII

Micrograms of thiamin excreted per day in the urine of patients with leukemia before and after the intravenous injection of 2-methyl-5-methoxyethyl-6-amino-pyrimidine

Patient	Number of days	Control period			After injection		
		Urinary thiamin range per day	Average per day	Highest per cent daily variation	24-hour output	Per cent increase over	
						Pre-vious day	Average control day
J. K.	5	238-403	327	69	164	59	-100
M. K.	2	70- 83	76	9	74	12	3
L. J.	4	44- 90	78	110	178	96	128
J. W.	3	65-215	165	230	170	26	3

cells would be at hand. From the results obtained in the previous section (B. 2.) of this communication, however, it is apparent that no retention of the vitamin exists. The leukemic patients excreted under normal conditions as much thiamin and PAYF as did the normal individuals.

DISCUSSION

It has been demonstrated in this investigation that leukemic blood cells are physiologically abnormal in their content and utilization of thiamin. This abnormality of leukemic cells is not the first which has been noted. Victor and Wintersteiner (7) found that both the aerobic and anaerobic glycolysis of leukemic leukocytes of mice were considerably higher than those of normal mice, but that no significant difference existed in the total respiratory activities (Q_{O_2}) of those cells.

Furthermore, when leukemic leukocytes are injected into normal mice, the leukocytes of the host also develop abnormal rates of carbohydrate metabolism before any morphological changes are recognizable (8). It also has been found that the blood of patients with leukemia has abnormal concentrations of substances concerned with enzyme systems, namely: choline esterase (9) and Coenzymes I and II (10). More recently, Butler and Cushman (4) have shown that the concentration of vitamin C in leukemic leukocytes and platelets was 10 times that of normal leukocytes and platelets.

From the results of the experiments presented, it appears that leukemic white cells no longer metabolize thiamin in the normal manner. This abnormality is a limitation in the conversion of the vitamin to the PAYF compound. The evidence for such a conclusion is (1) the PAYF of leukemic leukocytes and platelets forms an abnormally small percentage of the total thiamin present and (2) the administration of thiamin intravenously to 6 patients with the disorder always was followed by a decrease in the concentration of white cell thiamin; in 4 of 6 instances it was followed by a decreased concentration of PAYF and by urinary excretions of the vitamin significantly higher than those which followed the administration of an equal quantity of thiamin to normal individuals.

At present there is no apparent explanation for the limitation of thiamin conversion to PAYF and of the consequent accumulation of the intracellular vitamin. It has been accepted that the vitamin, in the form of a pyrophosphate, functions in a carboxylase enzyme system as a coenzyme (cocarboxylase). Thus a reasonable suggestion for its non-utilization by leukemic cells would be that in these cells (1) thiamin did not form the pyrophosphate ester or (2) the pyrophosphate was formed but was not used because of some inactivation of the enzyme itself.

Measurements of cocarboxylase in the whole blood of patients with leukemia have been made by Goodhart and Sinclair (3). Despite the fact that the patients studied were anemic, the concentration of cocarboxylase in their blood was found to be either within normal limits or elevated. Thus it would appear that there was no defect in the formation of cocarboxylase.

If it could be demonstrated that leukemic leukocytes were deficient in a carboxylase, or that the form in which that enzyme was present was altered, an explanation for the accumulation of thiamin or its pyrophosphate, cocarboxylase, would be at hand. To ascertain the facts concerning this question, it is necessary to devise methods for the quantitative estimation of a carboxylase in the white cells, and an attempt to do so is now under way in this laboratory.

Since the patient with leukemia does excrete normal amounts of PAYF in his urine, and in the same ratio to free thiamin as does the normal individual, it would appear that thiamin probably does undergo normal metabolic degradation elsewhere than in the blood cells. Furthermore, the metabolism of the vitamin in the leukocytes and platelets probably contributes but little to the total formation and urinary excretion of its breakdown product. The abnormal thiamin metabolism found in leukemic white cells probably does not exist to the same extent, or at all, in the other tissues of the patient.

CONCLUSIONS

1. The total thiamin levels in the leukocytes and platelets of patients with leukemia are about 3 times the normal average value, and in 82 per cent of the patients they are above the highest normal. The concentration of PAYF in the leukemic leukocytes and platelets forms an abnormally small percentage of the total thiamin content.

2. The total thiamin levels in the erythrocytes of leukemic patients are about twice the normal average level, and are above the highest normal range in 35 per cent of the patients examined. In the erythrocytes, however, the concentrations of PAYF formed a normal percentage of the total thiamin.

3. No obvious correlation was found to exist between the concentration of blood cell total thiamin and the form, severity or degree of associated leukocytosis of the leukemia, nor between the concentration and the sex or age of the patient.

4. No obvious relationship was found to exist between the white cell concentration of thiamin and the apparent youth of the cells.

5. Patients with leukemia excrete normal amounts of both thiamin and PAYF in the urine.

6. The probable explanation for the high concentration of white cell total thiamin is thought to be an impaired utilization of the thiamin, and not an increased ingestion or faulty excretion of the vitamin, nor the apparent youth of the cells involved.

7. Elevated blood cell concentrations of total thiamin have been found in patients with diseases other than leukemia; namely, with Hodgkin's disease and cancer of the gastro-intestinal tract, but not in patients with portal hepatic cirrhosis.

BIBLIOGRAPHY

1. Gorham, A. T., Abels, J. C., Robbins, A. L., and Rhoads, C. P., The measurement and metabolism of thiamin and of a pyrimidine stimulating yeast fermentation found in the blood cells and urine of normal individuals. *J. Clin. Invest.*, 1942, 21, 161.
2. Sinclair, H. M., Discussion on the clinical aspects of the vitamin B complex. *Proc. Roy. Soc. Med.*, 1939, 32, 812.
3. Goodhart, R., and Sinclair, H. M., Deficiency of vitamin B₁ in man as determined by blood cocarboxylase. *J. Biol. Chem.*, 1940, 132, 11.
4. Butler, A. M., and Cushman, M., Distribution of ascorbic acid in blood and its clinical significance. *J. Clin. Invest.*, 1940, 19, 459.
5. Warren, C. O., The Pasteur effect in bone marrow. *J. Cell. and Comp. Physiol.* (In Press.)
6. MacLeod, J., and Rhoads, C. P., Metabolism of leukocytes in Ringer phosphate and in serum. *Proc. Soc. Exper. Biol. and Med.*, 1939, 41, 268.
7. Victor, J., and Wintersteiner, M. R., Studies in mouse leukemia. X. Metabolic differences between transmission lines of mouse lymphatic leukemia. *Am. J. Cancer*, 1934, 22, 56.
8. Victor, J., and Potter, J. S., Influence of transmitted leukemia on metabolism of uninfected lymphoid tissue. *Brit. J. Exper. Physiol.*, 1938, 19, 227.
9. Sabine, J. C., Choline esterase of blood cells and plasma in blood dyscrasias with special reference to pernicious anemia. *J. Clin. Invest.*, 1940, 19, 833.
10. Vilter, S. P., Koch, M. B., and Spies, T. M., Coenzymes I and II in human blood. *J. Lab. and Clin. Med.*, 1940, 26, 31.

FURTHER STUDIES ON INCREASED SUSCEPTIBILITY TO CHLOROFORM POISONING PRODUCED IN THE ALBINO RAT BY INJECTION OF CRYSTALLINE THYROXIN

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In a previous article (1) evidence was presented of increased susceptibility to chloroform poisoning produced in the albino rat by injection of crystalline thyroxin. The present study was undertaken to obtain data that might throw light on the mechanism of this phenomenon, and was centered on two points. First: Is the increased susceptibility to chloroform poisoning in the hyperthyroid rat due to the fact that the glycogen content of the liver is diminished in the presence of hyperthyroidism? Second: Does a high protein diet afford the same protection against chloroform poisoning to the liver of a hyperthyroid rat that it does to the normal liver?

To test the hypothesis that a low glycogen content of the liver is responsible for the increased susceptibility to chloroform poisoning in rats following the administration of thyroxin, the problem was approached in two ways. In one set of experiments an attempt was made to maintain a normal glycogen content of the liver in animals receiving thyroxin, and their susceptibility to chloroform poisoning was then tested. In another series the liver glycogen was reduced by means other than administration of thyroxin, and the susceptibility to chloroform poisoning of this group was contrasted with that of animals whose liver glycogen had been reduced by administration of thyroxin.

METHOD AND MATERIALS

The experiments were carried out on male albino rats having an initial weight of between 150 grams and 175 grams.

The standard diet consisted of Purina Dog Chow (Ralston Purina Company). This was modified in some of the experiments by the addition of carbohydrate in the form of 6 grams of sucrose daily; and in others by the addition of 0.4 gram of Squibb's yeast or 3.0 grams of Fleischmann's dried irradiated yeast, number IF-39, daily (presented by the Fleischmann Laboratories). Each gram of Squibb's yeast contained 50 International units

B₁, 15 Sherman-Bourquin units B₂, 90 gamma B₆, 35 Jukes-Lepkovsky units Filtrate Factor, nicotinic acid. Each gram of Fleischmann's yeast contained 18 International units B₁, 20 Sherman-Bourquin units B₂.

In certain experiments a high protein diet was given, made up of Casein number 453 (Casein Company of America) 81.2 per cent, Crisco 7.2 per cent, McCollum salt mixture number 185 (2) 4.0 per cent, Vegex (Vitamin Food Company) 7.4 per cent, Squibb Navitol 0.3 per cent. (This is the high protein diet used by Goldschmidt *et al.* (3) and somewhat similar to that used by Moise and Smith (4).)

The thyroid hormone was given in the form of Squibb's crystalline thyroxin,¹ administered daily by subcutaneous injection over a period of approximately 2 weeks in doses of 0.1 mgm.²

The chloroform was injected subcutaneously in doses of 0.8 cc. per kgm. of rat, and was diluted with sterile mineral oil so that the total volume per dose was 0.5 cc.

Since we were interested only in the acute effect of chloroform poisoning, animals that survived were usually sacrificed at the end of 48 hours.

Animals that died were autopsied as soon as possible and blocks of tissue from their livers and other organs were fixed in Zenker's solution and in 4 per cent formalin. Animals that were sacrificed were killed by a sharp blow at the base of the skull and were autopsied in the same manner. Where glycogen determinations were to be carried out, the liver was removed at once—within 30 seconds. A portion of the liver was immediately fixed in absolute alcohol for later staining with Best's carmine. At the same time, duplicate portions of the liver weighing about 1 gram each were placed (for chemical determinations of the glycogen) in weighed tubes containing 30 per cent potassium hydroxide. The tubes were quickly heated in a water bath to initiate decomposition of the liver tissue, then removed for re-weighing, again returned to the water bath, and heated until the tissue had been completely dissolved. This process was followed by pre-

¹ The crystalline thyroxin used in these experiments was kindly contributed by E. R. Squibb and Sons.

² In the preliminary experiments 0.2 mgm. was given. With this dose a number of rats died; but since there was some respiratory infection among these rats, it was difficult to evaluate the relative importance of the infection and the larger dose of thyroxin. In later experiments, using the 0.1 mgm. dose, the animals in general remained in good condition.

precipitation of the glycogen with 95 per cent alcohol, according to the method of Good, Kramer and Somogyi (5). After hydrolysis, the glycogen was determined as glucose by a modification of the Hagedorn-Jensen technique (6).

EXPERIMENTAL

The animals were kept for a period of at least one week before experiments were begun in order to make sure they were all in healthy condition and to allow them to become adjusted to the laboratory environment. They were weighed every second day. In general their nutrition was well maintained. The control animals on standard diet uniformly gained in weight—usually 30 to 35 grams over the 2-week period. The animals receiving thyroxin usually suffered a loss of weight, although in many instances they maintained their weight or even made some gain. The rats often had a slight diarrhoea after the first thyroxin injections but generally remained in good condition.

Preliminary experiments. That the administration of the thyroid hormone results in a low level of glycogen in the liver was clearly demonstrated by the experiments of Cramer and Krause (7) in

TABLE I

The effect of the administration of crystalline thyroxin on the level of liver glycogen

It will be noted that the liver glycogen of animals receiving crystalline thyroxin over a period of from 11 to 17 days varied from 1.5 to 0.18 gram per cent, while in the control animals the level was from 5.0 to 7.1 grams per cent.

Rat number	Initial weight	Administration of crystalline thyroxin		Average daily food intake. Standard diet	Maximum weight	Final weight	Liver glycogen
		Total amount	Duration				
	grams	mgm.	days	grams	grams	grams	grams per cent
148	203	1.6	11	15	243	232	0.31
153	203	1.6	11	15	234	233	0.48
154	207	1.6	11	19	242	225	0.75
157	191	1.5	15	14	191	175	1.3
159	181	1.5	15	15	183	165	0.45
167	183	1.5	15	11	183	151	0.40
173	131	1.1	11	18	173	134	0.76
185	184	1.7	17	13	187	177	0.57
192	191	1.7	17	18	191	177	0.70
201	172	1.7	17	16	183	183	1.5
203	191	1.7	17	19	191	165	0.18
144	193	Control		17	219	219	6.9
145	207	Control		20	245	245	6.4
149	218	Control		18	243	243	7.1
152	192	Control		15	230	230	5.0

1913, and confirmed by Kuriyama (8, 9) in 1917. More recently, Coggeshall and Greene (10) have shown that a hyperthyroid animal has difficulty in storing glycogen in the liver.

The present experiments (Table I) show that a level of glycogen from 7.1 to 5.0 grams per cent was maintained in the group of control animals on the standard diet; whereas the level in animals receiving injections of crystalline thyroxin was from 1.5 to 0.18 gram per cent.

Attempts to maintain a normal level of liver glycogen in animals receiving injections of crystalline thyroxin. Kuriyama (9) found that if he gave a sufficiently high caloric diet to his thyroid-fed rats the liver glycogen was in some cases maintained to a limited extent. More recently, Drill (11) reported that rats receiving injections of crystalline thyroxin and large doses of yeast maintained a normal level of liver glycogen. Our own efforts along these lines were only partially successful. As shown in Table II, the addition to the diet of sucrose, either alone or combined with relatively small doses of yeast, did not appreciably alter the level of liver glycogen. When, however, large doses of yeast were added to the diet of rats Numbers 253, 255, 256, 258, 261, and 263 (Table II), the level of liver glycogen in 4 out of 6 instances was appreciably higher than in the rats not receiving this treatment.

Administration of chloroform to a group of animals that had received large doses of yeast in addition to their regular diet. A group of animals (Table III) treated by the same methods as rats Numbers 253, 255, 256, 258, 261, and 263, described above, were injected at the end of 14 days with 0.8 cc. of chloroform per kgm. of rat.³ Presumably, on the basis of the data given above, the majority of these animals had a liver glycogen level of about 2.5 grams per cent. It will be noted that only 2 of these rats survived. Three additional rats (Table III) that had previously been on a normal diet were given intraperitoneal injections of 5 per cent glucose at the time of the chloroform injections in an effort to increase

³ It had been found in earlier studies (1) that animals receiving this and somewhat larger doses of chloroform survived for more than 48 hours if they had not received preliminary thyroxin injections.

TABLE II

The effect on liver glycogen of additions of sucrose and varying amounts of yeast to the standard diet of hyperthyroid rats

It will be noted that the liver glycogen in rats that received sucrose, alone or combined with a small amount of yeast, was low; whereas the liver glycogen in 4 out of 6 rats that received a large amount of yeast was found to be at an appreciably higher level.

Rat number	Initial weight	Administration of crystalline thyroxin		Average daily food intake		Maximum weight	Final weight	Liver glycogen
		Total amount	Duration	Standard diet	Added sucrose or yeast			
	grams	mgm.	days	grams		grams	grams	grams per cent
198	173	1.6	16	19	6 grams sucrose	173	167	0.10
199	160	1.6	16	15	6 grams sucrose	173	173	0.11
200	203	1.6	16	16	6 grams sucrose	203	189	0.17
202	185	1.6	16	16	6 grams sucrose	187	183	0.11
183	187	1.6	16	15	6 grams sucrose and 0.4 grams yeast	193	193	0.16
193	176	1.6	16	9	6 grams sucrose and 0.4 grams yeast	181	179	0.42
196	197	1.6	16	12	6 grams sucrose and 0.4 grams yeast	197	187	0.11
197	157	1.6	16	9	6 grams sucrose and 0.4 grams yeast	173	168	0.12
165	192	1.2	13	19	0.4 grams yeast	192	183	0.68
175	178	1.2	13	19	0.4 grams yeast	178	169	0.83
182	179	1.7	17	16	0.4 grams yeast	179	173	0.28
186	162	1.7	17	14	0.4 grams yeast	174	171	1.1
189	189	1.7	17	15	0.4 grams yeast	197	187	0.43
190	195	1.7	17	17	0.4 grams yeast	197	194	0.49
253	179	1.2	12	13	3 grams yeast	180	160	2.6
255	196	1.2	12	16	3 grams yeast	196	185	0.83
256	182	1.2	12	14	3 grams yeast	199	199	2.9
258	199	1.2	12	15	3 grams yeast	199	181	2.6
261	173	1.2	12	14	3 grams yeast	178	159	0.43
263	214	1.2	12	14	3 grams yeast	214	189	1.7

the amount of glycogen in the liver; none of these rats survived.⁴

Comment. Since we were not able, by any of the methods tried, to obtain a completely normal level of liver glycogen, the evidence cannot be taken as conclusive that the increased susceptibility to chloroform poisoning shown by hyperthyroid rats is not connected with the low glycogen content of the liver found in these animals. The problem was therefore approached from another point of view. It was reasoned that, if the depletion of the liver glycogen were wholly or largely responsible for the increased susceptibility to chloroform poisoning in hyperthyroid animals, the same dose of chloroform would be equally

fatal to animals depleted of liver glycogen by other means. Experiments to test this hypothesis were undertaken.

Effects of chloroform injections on rats depleted of liver glycogen by fasting. It has been shown by Cori and Cori (12), Coggeshall and Greene (10), and Goldschmidt *et al.* (3) that withholding food for 24 to 48 hours will reduce the liver glycogen to a low level. The present experiments confirmed this finding, showing that the liver glycogen is reduced to a comparable, or in certain instances even a lower level, by this period of fasting than by the administration of thyroxin (*cf.* Tables IV and I).

To test the effects of chloroform injections on fasting rats as contrasted with hyperthyroid rats, 2 groups of animals were studied (Table V). One group, on the standard diet, received injections of crystalline thyroxin over a period of 12 to 16 days. The second group, also placed on the

⁴ It is interesting that Davis and Whipple (13) found that glucose given intravenously during chloroform anesthesia was of little benefit in protecting the liver against chloroform poisoning and may even have had a deleterious effect.

TABLE III

The effect of the administration of chloroform to 2 groups of rats treated with crystalline thyroxin

One group received 3.0 grams of yeast daily, in addition to the standard diet; the other group received intraperitoneal injections of glucose solution. It will be noted that in spite of these attempts to prevent depletion of the liver glycogen, only 2 of the 9 animals survived the chloroform injection.

Rat number	Initial weight	Administration of crystalline thyroxin		Average daily food intake		Dose of chloroform per kilo of body weight	Maximum weight	Final weight	Results
		Total amount	Duration	Standard diet	Yeast daily				
	<i>grams</i>	<i>mgm.</i>	<i>days</i>	<i>grams</i>	<i>grams</i>	<i>cc.</i>	<i>grams</i>	<i>grams</i>	
252	181	1.3	13	14	3	0.8	181	160	Dead in 24 hours
257	193	1.3	13	13	3	0.8	193	181	Dead in 24 hours
259	164	1.3	13	13	3	0.8	184	160	Dead in 24 hours
260	185	1.3	13	14	3	0.8	185	171	Dead in 28 hours
254	189	1.3	13	15	3	0.8	190	177	Survived
262	209	1.3	13	16	3	0.8	209	200	Survived
					5% Glucose cc.				
28	201	3.4	21	Not weighed	10*	1.5	233	233	Dead in 35 hours
41	189	4.3	15		10†	0.8	199	195	Dead in 45 hours
51	175	4.3	15		10‡	0.4	175	166	Dead in 20 hours

* One injection the day preceding administration of chloroform, 1 at the time of chloroform injection, and 1 the succeeding day.

† One injection the day preceding chloroform administration, 1 at the time of chloroform administration.

‡ One injection at the time of chloroform administration.

standard diet at the same time, received no food during the last 24 to 55 hours (water was freely available). At the end of the period, the animals of both groups were injected with 0.8 cc. of chloroform per kgm. of body weight. Among the 6 animals that had received preliminary treatment with thyroxin, there were 4 deaths; whereas among the 8 animals that had been deprived of food, there was only 1 death.

Comment. The question as to whether a high level of liver glycogen affords protection, either directly or indirectly, to the normal liver against chloroform poisoning, is still a moot point (3, 15).

The present experiments, however, show that depletion of liver glycogen by the administration of thyroxin is not the major factor in the increased susceptibility to chloroform poisoning shown by hyperthyroid rats.

Influence of a high protein diet. It has been reported by numerous investigators (3, 4, 13, 14) that a diet high in protein affords considerable protection to the normal liver from the injurious effects of chloroform. To ascertain whether or not this holds true in the presence of hyperthyroidism, a group of rats were placed on a diet containing approximately 81 per cent of protein

TABLE IV

The effect on liver glycogen of fasting for 24 to 48 hours

Contrast with Controls numbers 144, 145, 149 and 152, Table I; also with the group of animals receiving crystalline thyroxin, same Table.

Rat number	Initial weight	Experimental treatment	Weight at beginning of fast	Final weight	Liver glycogen
	<i>grams</i>		<i>grams</i>	<i>grams</i>	<i>grams per cent</i>
135	175	Food withheld 24 hours; water given freely	219		0.57
146	199	Food withheld 24 hours; water given freely	232	207	0.24
151	196	Food withheld 24 hours; water given freely	225	205	5.2*
147	207	Food withheld 48 hours; water given freely	242	199	0.23
155	190	Food withheld 48 hours; water given freely	199	188	0.26

* We have no explanation to offer for the normal reading in Animal number 151.

TABLE V

The effect of the injection of chloroform on animals that had received crystalline thyroxin in contrast with the effect on animals that had been deprived of food for 24 to 55 hours

It will be noted that 4 out of 6 of the thyroxin-injected animals died within 20 hours, while all but 1 of the animals from which food had been withheld survived.

Rat number	Initial weight	Administration of crystalline thyroxin		Average daily food intake. Standard diet	Dose of chloroform per kilo of body weight	Maximum weight	Final weight	Results
		Total amount	Duration					
	grams	mgm.	days	grams	cc.	grams	grams	
139	177	1.6	13	10	0.8	177	163	Dead in 18 hours
140	164	1.3	14	10	0.8	179	167	Survived
158	183	1.6	16	13	0.8	187	187	Dead in 15 hours
168	185	1.6	16	16	0.8	185	156	Dead in 15 hours
172	175	1.5	16	14	0.8	179	165	Dead in 20 hours
161	186	1.6	16	17	0.8	186	163	Survived
		Food withheld				Weight at beginning of fast		
		hours				grams		
136	161	24		10	0.8	181	170	Survived
137	152	24		11	0.8	189	173	Survived
142	153	24		12	0.8	181	162	Survived
143	150	48		11	0.8	191	179	Survived
156	170	48		12	0.8	181	145	Dead in 17 hours*
164	164	55		11	0.8	170	125	Survived
166	163	55		13	0.8	214	180	Survived
169	165	55		12	0.8	209	172	Survived

* Had not eaten well for several days preceding fast.

for a period of 2 weeks, during which time they received daily injections of crystalline thyroxin. A control group of 6 rats received the same diet but no thyroxin. At the end of 15 days both

groups received an injection of 0.8 cc. of chloroform per kgm. of rat. The results are shown in Table VI. It will be noted that all the animals that had received thyroxin plus a high protein

TABLE VI

The effect of chloroform injections on 2 groups of animals fed on a high protein diet

It will be noted that this diet did not afford protection against chloroform poisoning to animals receiving crystalline thyroxin.

Rat number	Initial weight	Administration of crystalline thyroxin		Average daily food intake. Casein diet	Dose of chloroform per kilo of body weight	Maximum weight	Final weight	Results
		Total amount	Duration					
	grams	mgm.	days	grams	cc.	grams	grams	
240	215	1.5	15	14	0.8	215	175	Dead in 18 hours*
241	199	1.5	15	10	0.8	200	175	Dead in 42 hours*
242	190	1.5	15	11	0.8	195	165	Dead in 24 hours*
243	209	1.5	15	11	0.8	211	170	Dead in 21 hours*
244	209	1.5	15	16	0.8	209	179	Dead in 21 hours*
245	199	1.5	15	11	0.8	207	161	Dead in 20 hours*
230	226	Control		12	0.8	226	224	Survived 48 hours
231	259	Control		19	0.8	259	235	Survived 48 hours
232	276	Control		12	0.8	278	278	Survived 48 hours
236	259	Control		8	0.8	259	236	Survived 48 hours
238	221	Control		12	0.8	221	217	Survived 48 hours
250	226	Control		15	0.8	226	201	Survived 48 hours†

* Showed histological evidence of extensive liver damage.

† Moribund at 48 hours.

diet died within 42 hours, all but 1 dying within 24 hours;⁵ whereas the animals that did not receive thyroxin all survived 48 hours, at which time they were sacrificed.

Comment. The exact mechanism by which a high protein diet affords protection to the normal liver against chloroform poisoning is still a matter of some conjecture. Whatever the correct interpretation, it is of some interest that this protection is not afforded in the presence of hyperthyroidism.

Histological studies. The changes found at autopsy in the liver and other organs of the animals that had received thyroxin followed by chloroform injections have been described in a previous report (1). In the present studies, similar changes were found in animals that were autopsied, the liver lesions consisting in varying degrees of central necrosis, often extreme. In considering the effects of chloroform, it should be borne in mind that Goodpasture (16) found, in experiments on rabbits which had been receiving thyroid hormone, that when small doses of chloroform were given by inhalation, the principal pathological changes occurred in the myocardium; whereas when the chloroform was injected subcutaneously, the greatest effect was upon the liver.

CONCLUSIONS

1. Our data do not furnish support of the view that the increased susceptibility to chloroform poisoning in the hyperthyroid rat is due chiefly to the low level of liver glycogen found in these animals.

2. A high protein diet does not afford protection against chloroform poisoning to the liver of animals receiving thyroid hormone.

BIBLIOGRAPHY

- McIver, M. A., Increased susceptibility to chloroform poisoning produced in the albino rat by injection of crystalline thyroxin. *Proc. Soc. Exper. Biol. and Med.*, 1940, 45, 201.
- McCollum, E. V., and Simonds, N., A biological analysis of pellagra-producing diets. II. The minimum requirements of the two unidentified dietary factors for maintenance as contrasted with growth. *J. Biol. Chem.*, 1917, 32, 181.
- Goldschmidt, S., Vars, H. M., and Ravdin, I. S., The influence of the foodstuffs upon the susceptibility of the liver to injury by chloroform and the probable mechanism of their action. *J. Clin. Invest.*, 1939, 18, 277.
- Moise, T. S., and Smith, A. H., Diet and tissue growth. I. The regeneration of liver tissue on various adequate diets. *J. Exper. Med.*, 1924, 40, 13.
- Good, C. A., Kramer, H., and Somogyi, M., The determination of glycogen. *J. Biol. Chem.*, 1933, 100, 485.
- Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry. Vol. II. Williams and Wilkins, Baltimore, 1932, p. 471.
- Cramer, W., and Krause, R. A., Carbohydrate metabolism and its relation to the thyroid gland. *Proc. Roy. Soc. London, s.B.*, 1913, 86, 550.
- Kuriyama, S., The influence of thyroid feeding upon carbohydrate metabolism. *Am. J. Physiol.*, 1917, 43, 481.
- Kuriyama, S., The influence of thyroid feeding upon carbohydrate metabolism. I. The storage and mobilization of the liver glycogen in thyroid-fed animals. *J. Biol. Chem.*, 1918, 33, 193.
- Coggeshall, H. C., and Greene, J. A., The influence of desiccated thyroid gland, thyroxin, and inorganic iodine upon the storage of glycogen in the liver of the albino rat under controlled conditions. *Am. J. Physiol.*, 1933, 105, 103.
- Drill, V. A., The effect of yeast on the liver glycogen of white rats during hyperthyroidism. *J. Nutrition*, 1937, 14, 355.
- Cori, C. F., and Cori, G. T., The fate of sugar in the animal body. II. The relation between sugar oxidation and glycogen formation in normal and insulinized rats during the absorption of glucose. *J. Biol. Chem.*, 1926, 70, 557.
- Davis, N. C., and Whipple, G. H., The influence of fasting and various diets on the liver injury effected by chloroform anesthesia. Paper I. *Arch. Int. Med.*, 1919, 23, 612.
- Miller, L. L., and Whipple, G. H., Chloroform liver injury increases as protein stores decrease. Studies in nitrogen metabolism in these dogs. *Am. J. M. Sc.*, 1940, 199, 204.
- Davis, N. C., and Whipple, G. H., The influence of drugs and chemical agents on the liver necrosis of chloroform anesthesia. Paper II. *Arch. Int. Med.*, 1919, 23, 636.
- Goodpasture, E. W., The influence of thyroid products on the production of myocardial necrosis. *J. Exper. Med.*, 1921, 34, 407.

⁵ The substitution of a small amount of a mixture of dextrose and maltose (Karo Corn Syrup) for a part of the Casein and Crisco in the high protein diet seems to increase somewhat the resistance of hyperthyroid rats to chloroform poisoning; but our experiments on this point were too few in number to be taken as conclusive.

THE EFFECTS OF LARGE INTRAVENOUS INFUSIONS ON BODY FLUID¹

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Much consideration has been given to the changes which result in man and in experimental animals from administering by vein various quantities of fluids of different composition (1, 2, 3). Thus, the dislocation of body fluid and the urinary changes which follow rapid infusion of massive quantities of fluid in animals have been described (4, 5, 6). Shifts of water and salts between muscle and blood after infusion of isotonic fluids of varying pH have been studied in dogs by means of muscle biopsy and analysis (7). The circulatory effects of administering fluid by vein in various clinical conditions have been investigated, having in mind the primary importance of circulatory dynamics in determining response to intravenous fluid therapy (1, 8). Such studies have served to emphasize the fundamental conclusions of Gamble (9, 10, 11), Peters (12, 13), and others.

In the work on which the present report is based it seemed desirable to determine the results of large amounts of isotonic glucose and NaCl solutions administered by constant intravenous drip over a period of several days. The physiological responses to sustained submaximal infusions were under inquiry rather than the reaction to a large or small intravenous injection of brief duration, for it seemed possible that compensatory mechanisms of a different nature might be brought into play as the infusions were continued.

MATERIAL AND METHODS

Patients undergoing mild or moderately severe operations under ether anesthesia were studied just before operation, just after operation, at the end of the infusion period, and several days afterwards when the effects of the infusion had subsided. Determinations were made on a fasting basis, and the patients took water as desired during the infusion period but were allowed only small amounts of fruit juices in addition. The patients

were well-nourished women without cardiovascular or renal disease. During the study they were under constant observation by trained attendants and, if a patient objected, the infusion was discontinued. Urine was collected quantitatively by an indwelling catheter. Arm or leg veins were used for the infusion, the extremity being splinted, and except for slight redness about the needle in two or three instances no local reaction was noted. The patients were entirely afebrile, or nearly so, during the study. Intake of fluid by oral and intravenous routes and output of urine were totaled at 12-hour intervals. Four patients received 5 per cent glucose solution and six 0.9 per cent NaCl solution.

ANALYTICAL METHODS

Blood loss at operation was determined by the method of Gatch and Little (14), sodium of serum or plasma and of urine by the gravimetric technique of Butler and Tuthill (15), potassium by the method of Fiske and Litarczek (16), chloride by Wilson and Ball's method (17), carbon dioxide content of serum according to Van Slyke and Sendroy (18), total nitrogen by macro-Kjeldahl (19) on oxalated plasma or serum and urine, and non-protein nitrogen of serum or plasma by micro-digestion and nesslerization. Serum albumin was determined by Howe's method (20). From the total protein of the serum and the serum albumin, colloid osmotic pressure ("oncotic pressure") was calculated, using the nomographic formula of Wells, Youmans, and Mills (21). Oxygen capacity was determined on heparinized venous blood drawn without stasis and equilibrated with room air at room temperature (22). Hematocrit readings were made by adding 4 cc. of blood to 1 cc. of 1.1 per cent sodium oxalate and centrifuging in hematocrit tubes until no further change in the reading occurred, precautions being taken against loss of carbon dioxide. In some of the patients receiving 5 per cent glucose by vein the urinary excretion of glucose was measured, but these data are not included as the losses were trivial.

Plasma volume was determined by the method of Gregersen and his coworkers (23), using the blue dye T-1824, the serum concentrations being measured spectrophotometrically. "Available fluid" volume, taken as extracellular fluid volume plus the water of red blood cells, was determined as described by Stewart and Rourke (24). The patients were weighed on a stretcher fitting over silk scales which were accurate to within 10 grams.

¹ Aided by grants from the William F. Milton Fund, Harvard University, and the Josiah Macy, Jr. Foundation.

RESULTS

Data are presented as obtained in three of the more conclusive experiments. Patient R. H. who underwent colporrhaphy and uterine suspension under ether anesthesia received 26.69 liters of 0.9 per cent NaCl solution intravenously during a period of 96 hours, beginning immediately after operation. Convalescence was uneventful, and the patient seemed to have less than usual post-operative discomfort. There was no detectable edema at any time. Patient G. S., following colporrhaphy under ether anesthesia, received intravenously during a period of 144 hours 28.37 liters of 5 per cent glucose solution without any untoward clinical effects and without edema. Patient S. K., following a trivial surgical procedure consisting of uterine dilatation and curettage under ether anesthesia, was given 6.86 liters of 5 per cent glucose solution by vein during a 36-hour period. Signs of water intoxication appeared, including nausea and vomiting, epigastric distress, disorientation and excitement, culminating shortly in coma. The infusion was immediately discontinued and complete recovery occurred in a few hours without the need of giving salt solution. Convalescence was subsequently uninterrupted. The patient was a young woman, 20 years old, in

good general condition and without detectable renal, cardiovascular or hepatic disease.

In Figure 1 are shown fluid intake and urine volume measurements in the three experiments, together with body weight before and after the infusion. Measurements of intake and urine volume do not, of course, permit complete appraisal of the state of water balance, for water of oxidation on the one hand and extrarenal loss of water on the other are not considered. Since the latter factors are roughly stationary, however, the values do show the trend of water balance. Comparison of the G. S. and S. K. data bring out clearly the lag in water removal, which together with lack of sodium conservation (Figure 4) produced a rapid reduction of plasma sodium concentration in patient S. K.

In Figure 2 are shown balance data with respect to sodium, chloride ion and potassium, as found in patient R. H. The record of cumulative sodium balance brings out the slowness with which the mechanisms governing extracellular fluid volume operate against an inflow of isotonic NaCl solution. For 4 days they succeed only in progressively reducing the rate of increment, but by the eighth period they finally have the upper hand. The tolerance which the body shows for increase

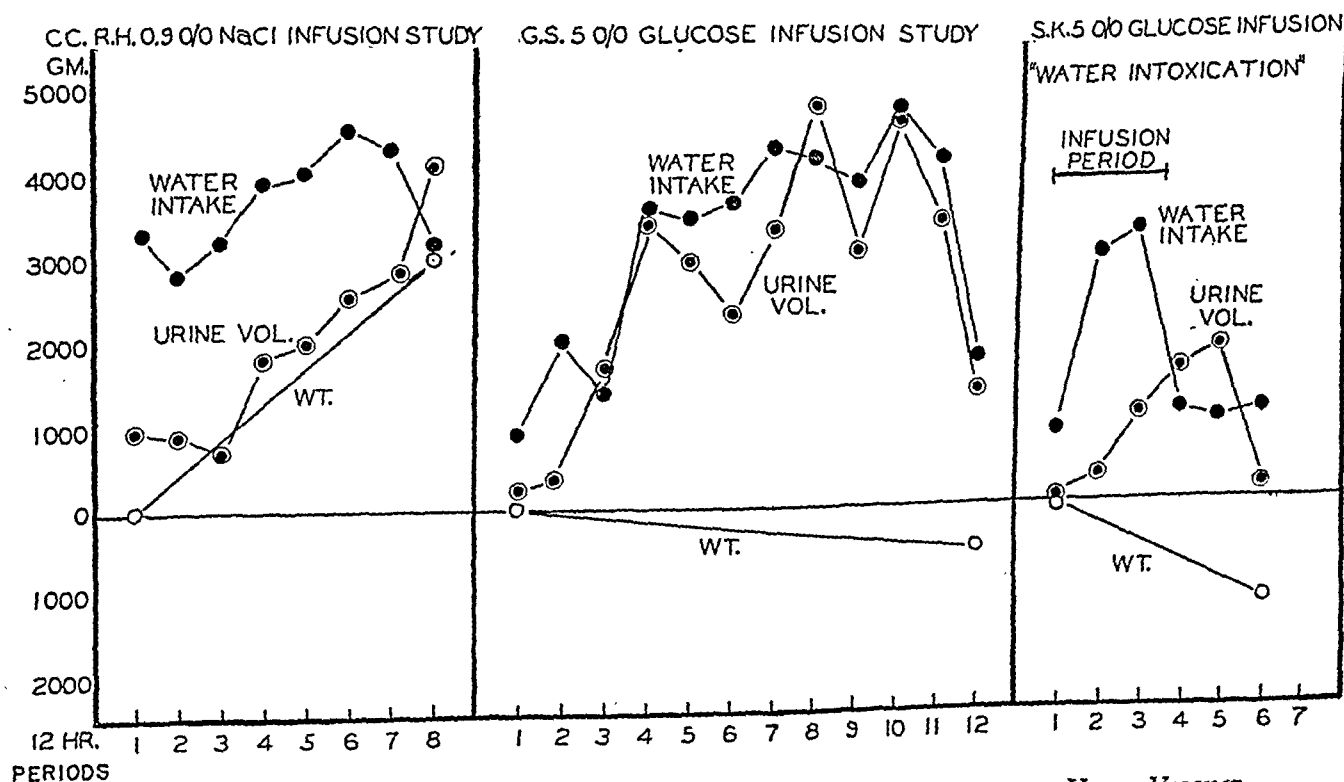


FIG. 1. MEASUREMENTS OF BODY WEIGHT CHANGE, TOTAL WATER INTAKE AND URINE VOLUME

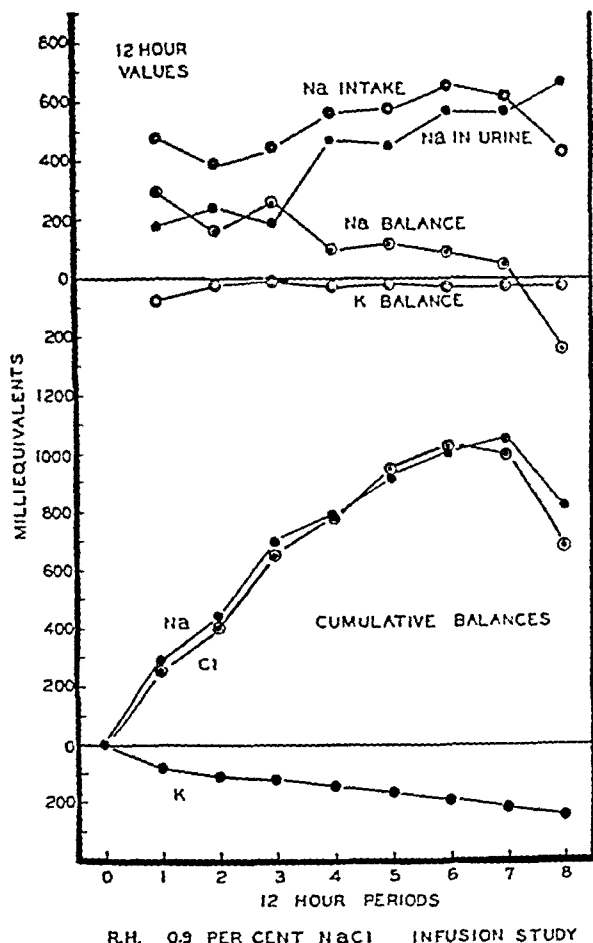


FIG. 2. ELECTROLYTE BALANCE DATA FROM SALT INFUSION STUDY

in fluid when sodium and chloride ion are supplied with water is also indicated by the values shown in the upper half of the figure.

In Figure 3 are recorded the incidental losses of sodium, chloride ion, potassium and nitrogen. The scale of the nitrogen ordinate in grams is adjusted to that of the milliequivalent ordinate in terms of the nitrogen:potassium content of protoplasm (muscle).

The protein-sparing effect of glucose is shown by the more gradual loss of nitrogen for G. S. as compared with R. H. The loss of potassium in excess of nitrogen indicates withdrawal of intracellular fluid. The nitrogen-potassium discrepancy is much larger in the NaCl than in the glucose study. The total potassium loss for R. H. is 240 milliequivalents, while on the basis of nitrogen excretion 110 milliequivalents derive

from destroyed protoplasm ($44 \times 2.5 = 110$). The remaining 130 milliequivalents correspond to 950 cc. of intracellular fluid. Such a relatively small loss from the body's total content of intracellular fluid is probably not physiologically significant. The discrepancy in the nitrogen-potassium elimination appears to be largely an initial event occurring in the early postoperative period. It is probably dependent to some extent on anesthesia and surgical trauma.

The loss of sodium over the 6 days of the glucose infusion in the case of patient G. S. was 289 milliequivalents. On the basis of the usual sodium content of interstitial fluid, 147 milliequivalents per liter, this implies a loss of 1960 cc. Total extracellular fluid for this patient estimated as 20 per cent of body weight is 13,660 cc. The loss would therefore amount to 14.5 per cent, a considerable but not a seriously large loss. In the case of S. K., however, a rapid loss of sodium from failure of renal conservation, together with the lag in water removal (Figure 1), produced a reduction of sodium concentration to a dangerous extent.

Figure 4 presents data on concentration of sodium in urine and plasma. The urine data from the NaCl study produce an impressive curve, which would seem to show quite clearly that the progressive decline in the rate of increase in extracellular fluid volume and the eventual reduction of volume are accompanied by an increasing removal of water with respect to sodium. The data from the glucose study (Patient G. S.) show very strikingly how extensive is the ability of the kidney to dilute sodium and how nearly the theoretically required zero excretion is approached. An approximately thirty-fold dilution of sodium with respect to its plasma level is attained. The data obtained from Patient S. K., in contrast with those from G. S., make clear the failure of this individual to conserve sodium in the presence of a large water excretion. In possible explanation it may be noted that 6867 cc. were given as compared with 3005 cc. for Patient G. S. over the first 30 hours of the infusion period, although thereafter this patient received approximately 6 liters per 24 hours without untoward effects. As seen in Figure 4, the urine

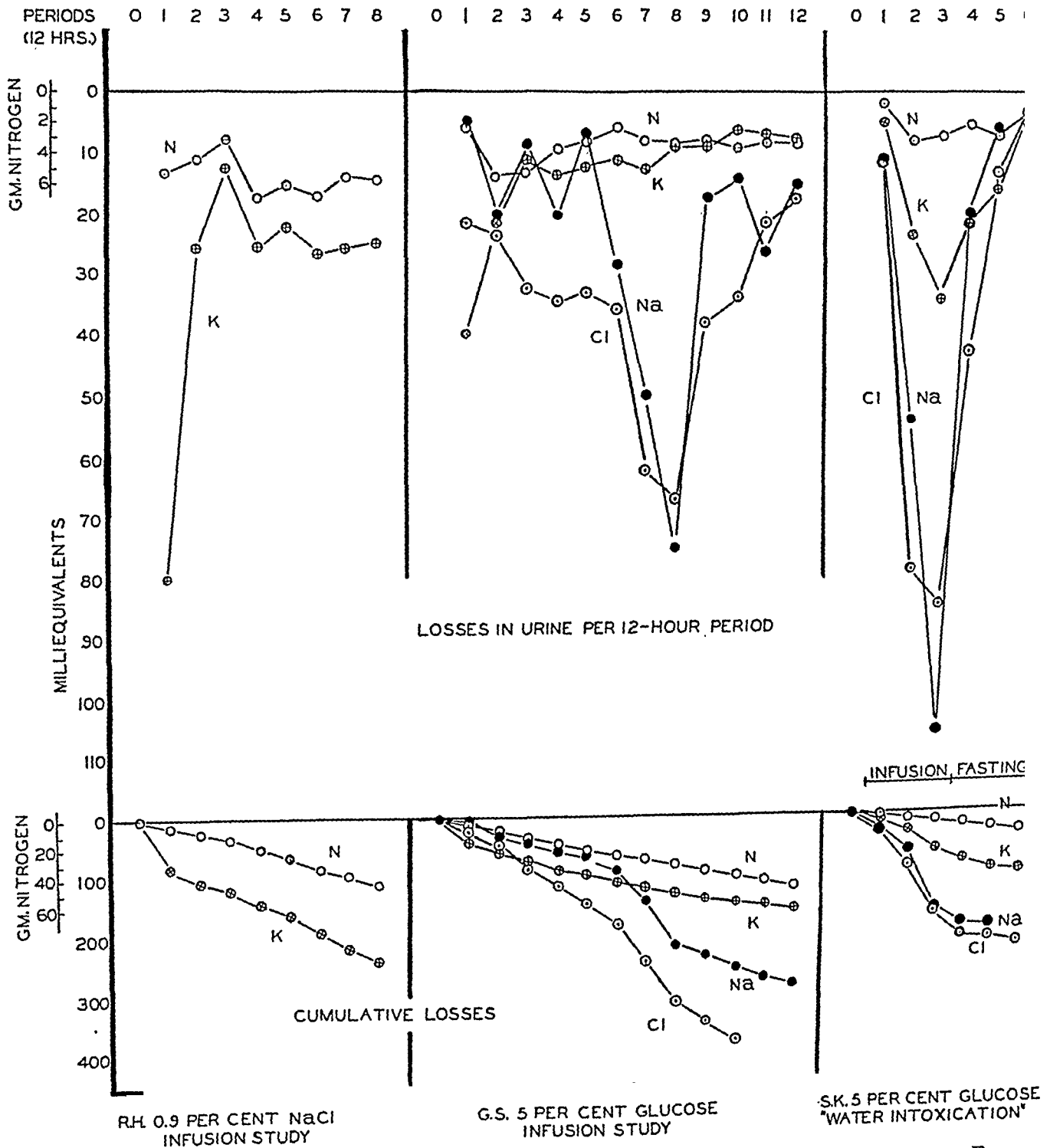


FIG. 3. MEASUREMENTS OF LOSSES OF NITROGEN, SODIUM, CHLORIDE ION AND POTASSIUM INCIDENTAL TO FASTING

sodium concentrations for S. K. progressively decline during the period of water intoxication and with cessation of infusion fall at once to the extremely low level found for G. S. The data thus suggest that the load suddenly placed on renal regulation is larger than can be immediately managed.

Tables I, II and III show data obtained in study-

ing the concentration of various factors in the blood during the progress of the experiments. "Available fluid" volume and plasma volume were also determined. The accuracy with which normal values are re-established after cessation of the infusion is strikingly shown in Table I. Table IV presents balance data for the infusion period in the three cases.

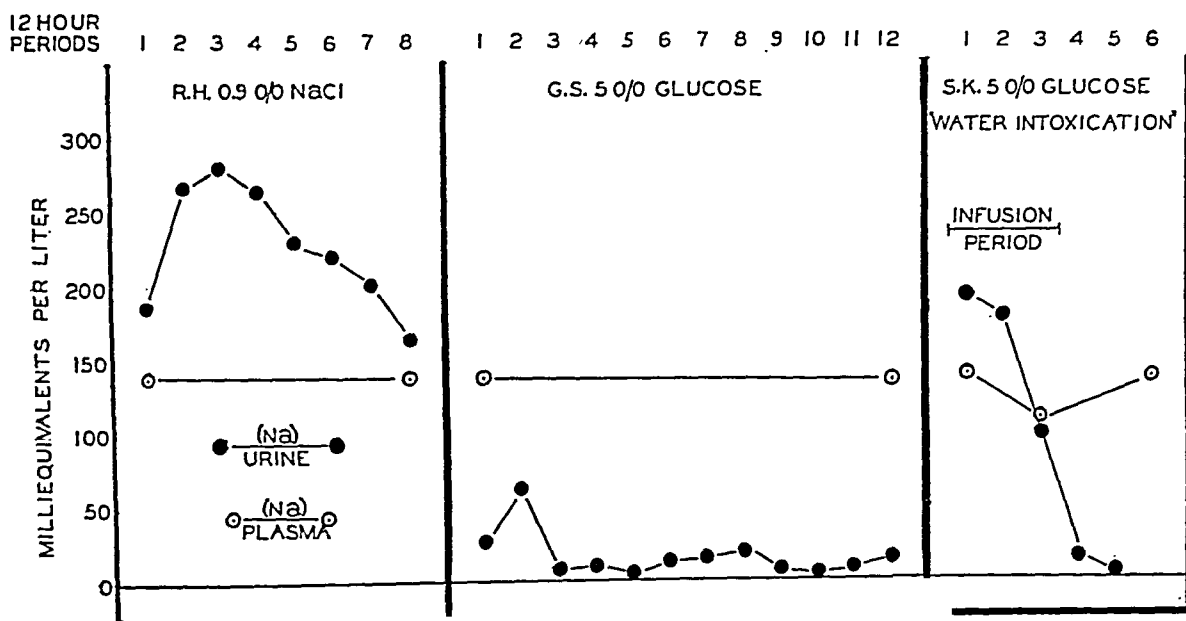


FIG. 4. CONCENTRATIONS OF SODIUM IN URINE AND IN PLASMA

DISCUSSION

For the purposes of the experimental plan the patients studied were normal, healthy individuals, who were making uncomplicated recovery from moderate surgical trauma and ether anesthesia. As for the disturbances in body fluid connected with the anesthesia and operation in this group of cases, pertinent data have already been reported (25). At no time during the infusion did any of the patients show gross evidence of edema, a rather remarkable fact inasmuch as huge addi-

tions to body fluid were made in the saline infusion studies. It is of interest also that the sensation of thirst was present, being perhaps more constant in the patients who were receiving glucose infusions.

These studies show sharp differences between the effects of isotonic sodium chloride solution and isotonic glucose solution when such solutions are given at a constant rate by vein over a period of several days. In general terms the saline solution tends to be retained, with consequent increase

TABLE I

	Preoperative	Postoperative	Infusion stopped	Recovered
Date.....	May 19	May 19	May 24	May 28
Weight, <i>kgm.</i>	60.49	59.95	67.23	58.18
Hematocrit, <i>per cent.</i>	42.8	41.9	25.6	33.3
Oxygen capacity, <i>volume per 100 cc.</i>	16.3	16.8	11.9	13.6
total, <i>cc.</i>	841	869	757	633
Plasma protein, <i>grams per 100 cc.</i>	8.16	7.76	5.97	7.82
total, <i>grams</i>	246	233	283	243
Plasma A-G ratio.....	1.39	1.47	1.22	1.04
Serum oncotic pressure, <i>mm. H₂O.</i>	410	392	247	350
Serum Na, <i>m. eq. per liter.</i>	136.2	139.9	138.5	134.0
Serum K, <i>m. eq. per liter.</i>	3.9	3.9	3.8	4.6
Serum Cl, <i>m. eq. per liter.</i>	102.5	106.5	110.0	100.0
Serum HCO ₃ , <i>m. eq. per liter.</i>	26.8	24.7	22.4	29.2
Plasma volume, <i>cc.</i>	3013	3008	4730	3106
"Available fluid" volume, <i>cc.</i>	12130	11960	21850	13970
Serum NPN, <i>mgm. per 100 cc.</i>	24.4	27.2	12.5	33.0

R. H.—Infusion study, 0.9 per cent NaCl solution, 26.69 liters given. Operation consisting of colporrhaphy and uterine suspension, ether anesthesia. Blood loss 357 cc.

TABLE II

	Preoperative	Postoperative	Infusion stopped
Date.....	June 8	June 8	June 14
Weight, <i>kgm.</i>	69.83	68.35	67.88
Serum protein, <i>grams per 100 cc.</i>	7.58	7.47	7.60
Serum NPN, <i>mgm. per 100 cc.</i>	21.2	19.2	15.0
Serum Na, <i>m. eq. per liter</i>	137.5	137.3	133.4
Serum Cl, <i>m. eq. per liter</i>	106.0	106.0	91.0

G. S.—Postoperative infusion study, 5 per cent glucose solution, 28.37 liters given. Operation consisting of colporrhaphy under ether anesthesia.

in plasma and extracellular fluid volume, dilution of plasma protein, and dilution of red blood cells. After a period of several days a maximal point is reached, the excretory mechanisms succeed in turning the tide, and urine volume equals or exceeds infusion volume. On the other hand, the infusion of isotonic glucose solution sets into

TABLE III

	Preoperative	Postoperative	Hydrated
Hematocrit, <i>per cent</i>	37.5	37.9	35.1
Oxygen capacity, <i>volume per 100 cc.</i>	16.3	16.0	15.0
Plasma protein, <i>grams per 100 cc., total, cc.</i>	714	785	761
Plasma A-G ratio	7.66	7.67	7.20
Serum oncotic pressure, <i>mm. H₂O</i>	209	234	237
Serum Na, <i>m. eq. per liter</i>	1.54	1.59	1.25
Serum K, <i>m. eq. per liter</i>	376	380	330
Serum Cl, <i>m. eq. per liter</i>	140.5	138.3	112.0
Serum HCO ₃ , <i>m. eq. per liter</i>	4.4	3.6	3.1
Plasma volume, <i>cc.</i>	105.0	105.0	86.5
"Available fluid," <i>volume, cc.</i>	26.8	19.1	21.2
Serum NPN, <i>mgm. per 100 cc.</i>	2736	3050	3290
	11810	11180	15380
	23.4	23.1	13.2

S. K.—Infusion study, 5 per cent glucose solution, duration 28 hours. Operation consisting of uterine curettage under ether anesthesia, blood loss 65 cc. Water intoxication and recovery.

immediate activity protective mechanisms through which the kidney defends the electrolyte com-

TABLE IV
Balance data

Study	12-hour period	Intake					Urine				
		H ₂ O intra-venous	H ₂ O post-operative	H ₂ O total	NaCl	Glucose	Volume	Na	Cl	K	N
I 0.9 per cent NaCl, Subject R. H.		<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>mEq.</i>	<i>grams</i>	<i>cc.</i>	<i>mEq.</i>	<i>mEq.</i>	<i>mEq.</i>	<i>grams</i>
	1	3,080	225	3,305	476		940	178	222	79	5.34
	2	2,545	270	2,815	393		880	236	246	25	4.47
	3	2,850	360	3,210	441		670	186	188	12	2.91
	4	3,615	330	3,945	559		1,760	465	422	25	6.92
	5	3,700	330	4,030	572		1,960	453	419	22	6.23
	6	4,224	360	4,584	653		2,540	564	574	26	6.73
	7	3,940	360	4,300	609		2,820	563	635	26	5.61
	8	2,740	360	3,100	423		4,065	666	739	25	5.70
II 5 per cent Glucose, Subject G. S.	Total	26,694	2,595	29,289	4,126		15,635	3,311	3,445	240	43.91
	1*	840		840		42	220	6	21	40	2.43
	2	1,135	570	2,005		57	325	21	23	21	5.77
	3	1,030	285	1,315		51	1,675	9	33	11	4.96
	4	2,530	1,050	3,580		126	3,480	20	35	13	3.83
	5	2,785	705	3,490		139	2,940	7	33	12	3.23
	6	2,565	1,080	3,645		128	2,325	28	36	11	2.44
	7	3,465	885	4,350		173	3,380	50	62	13	3.19
	8	3,213	1,080	4,293		160	4,735	75	67	8	3.41
	9	3,310	540	3,850		165	3,075	17	38	9	3.21
	10	3,702	1,080	4,782		185	4,690	14	34	6	3.79
	11	3,325	900	4,225		165	3,440	27	21	7	3.30
	12	475	1,350	1,825		23	1,365	15	18	8	3.43
III 5 per cent Glucose, Subject S. K.	Total	28,375	9,525	37,900		1,414	31,650	289	421	159	42.99
	1*	750	60	810		37	70	12	11	6	0.78
	2	2,850	165	3,015		142	305	54	79	24	3.31
	3	3,267	45	3,312		163	1,040	106	85	34	3.08
	Total	6,867	270	7,137		342	1,415	172	175	64	7.17
	4	0	60	60			1,545	21	43	22	2.33
	5	0	0	0			1,820	7	24	15	3.00

* Six-hour period.

position of the extracellular fluids, and large amounts of urine are excreted containing extraordinarily little sodium and chloride. Dilution of plasma colloids and formed elements in the blood is accordingly averted. Putting it differently, the renal regulator is extremely sensitive to changes in composition of extracellular fluid, but is quite tolerant of increases in extracellular fluid volume.

It is evident that the administration of isotonic glucose solution by vein in the fasting subject results in reduced consumption of body protoplasm through the protein-sparing action of glucose. Such weight-conserving effects of glucose infusion are offset by the reduction in extracellular fluid stores accompanying the loss of sodium and chloride ion in the urine. The remarkable power of the healthy kidney to dilute sodium and chloride ion minimizes but does not entirely prevent the dehydrating action of isotonic glucose infusions. As shown in the study of Patient S. K., a dangerous body fluid disturbance may occur during the infusion of large amounts of isotonic glucose solution. The two factors which brought on the syndrome of water intoxication in this patient were retention of water without electrolyte, and failure in renal conservation of sodium and chloride ion.

A finding of interest was the mobilization of plasma protein by the saline infusion, but not at all, or much less extensively, by the glucose infusion. It would appear that reserve protein in the amount of 25 per cent or so of that in the plasma may be placed in circulation under conditions which tend to dilute plasma colloid, and apparently the loan is accurately paid back after the period of stress. If reliance can be placed on the albumin-globulin partition by the method used, then the reserve protein appears to be predominantly globulin. Nevertheless, the plasma benefits by an important increment in colloid osmotic pressure as a result of such additions. Presumably, large increases in plasma and extracellular fluid volume, as brought about by saline infusions, would be accompanied by increases in lymph and, if so, the total amount of extravascular circulating protein might also be augmented. It is a notable fact that the addition of plasma protein to the circulation taking place during saline infusions was unaccompanied by an increase in total circulating hemoglobin.

In considering values for extracellular fluid volume in these studies, it should be borne in mind that the thiocyanate ion is taken up by the water of red blood cells and, further, that at diffusion equilibrium between thiocyanate in serum and in transudates, the concentration in transudates is about 100/110 of that in the serum (26). Consequently, the "available fluid for solution of thiocyanate" is to be regarded as only a rough approximation of true extracellular fluid volume, and the determination is of chief interest in showing changes in the same individual under varying conditions.

CONCLUSIONS

According to the data presented from three patients who underwent simple surgical procedures involving only slight loss of extracellular fluid, the chief effects of continued infusion of 0.9 per cent sodium chloride solution and of 5 per cent glucose solution on a basis of fasting are as follows:

Salt solution. Continuous infusion to the extent of 6.5 liters daily produces a very large addition to the volume of extracellular fluid, amounting to approximately 80 per cent of the initial volume. The daily increments, however, become progressively smaller and at the end of 3 to 4 days a maximum expansion of volume is reached which is followed by a gradual process of subsidence. In terms of initial volumes, the increase of interstitial fluid is approximately 90 per cent and that of blood plasma 60 per cent. An addition to the quantity of protein in plasma occurs to an extent which provides a protein concentration at nearly 6 grams per 100 cc. The circulation of extracellular fluid between the vascular and interstitial compartments is thus sustained and edema does not develop. Comparing the loss of nitrogen and potassium incidental to fasting with the N:K ratio in muscle tissue, a relatively small withdrawal of intracellular fluid, 950 cc., is indicated.

The findings describe the surprising extent to which the extracellular fluid compartments can be overfilled without apparent evidence of functional disturbance.

Glucose solution. In contrast with the effect of salt solution, continuous infusion of 5 per cent

glucose solution causes actual reduction of the volume of extracellular fluid, as evidenced by loss of body weight and of sodium. This dehydrating effect is referable to a not quite complete conservation of sodium by the kidney. Renal effectiveness in controlling the formation of an enormous quantity of urine (5 liters daily) under circumstances which severely test the reabsorptive mechanisms is, however, very remarkable. The concentration of sodium in the urine is held below 0.01 M and sodium concentration in the plasma is almost completely sustained. A small protein-sparing effect from the glucose is shown by a 30 per cent reduction in the rate of nitrogen loss found in the salt infusion study. According to the relative quantities of nitrogen and potassium excreted in the urine, there is no appreciable withdrawal of intracellular fluid. The initial rate of glucose infusion was 2.4 liters per 24 hours. This was increased on the second day to approximately 6 liters.

According to evidence from a single patient, infusion of glucose solution at an initial rate of 5.5 liters per 24 hours may cause derangement of renal control of the electrolyte structure of the plasma (water intoxication). This is shown by a failure to conserve sodium and a consequent rapid fall of sodium concentration in the plasma. With cessation of infusion there is immediate recovery of renal control. The concentration of sodium falls in the urine and rises to its usual level in the plasma. The data clearly suggest overtaking of the capacity of renal regulation.

These findings illustrate the fallacy of regarding glucose solution as a means of replacing losses of extracellular fluid and indicate the possible harmfulness of large infusions. They do not disturb the rationale of moderate amounts of glucose solution given intravenously to support renal function by providing a physiologically suitable volume of urine and to replace insensible expenditures of water.

Data in agreement with those presented were obtained from two patients given glucose solution and from five receiving salt solution.

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BIBLIOGRAPHY

1. Murphy, F. D., Correll, H., and Grill, J. C., The effects of intravenous solutions on patients, with and without cardiovascular defects. *J. A. M. A.*, 1941, 116, 104.
2. Blalock, A., Beard, J. W., and Thuss, C., Intravenous injections. A study of the effects on the composition of the blood of the injection of various fluids into dogs with normal and with low blood pressure. *J. Clin. Invest.*, 1932, 11, 267.
3. Coller, F. A., Dick, V. S., and Maddock, W. G., Maintenance of normal water exchange with intravenous fluids. *J. A. M. A.*, 1936, 107, 1522.
4. Cutting, R. A., Lands, A. M., and Larson, P. S., Distribution and excretion of water and chlorides after massive saline infusions. *Arch. Surg.*, 1938, 36, 586.
5. Warthen, H. J., Massive intravenous injections. *Arch. Surg.*, 1935, 30, 199.
6. Cutting, R. A., Larson, P. S., and Lands, A. M., Cause of death resulting from massive infusions of isotonic solutions. *Arch. Surg.*, 1939, 38, 599.
7. Hastings, A. B., and Eichelberger, L., The exchange of salt and water between muscle and blood. I. The effect of an increase in total body water produced by the intravenous injection of isotonic salt solutions. *J. Biol. Chem.*, 1937, 117, 73.
8. Gilligan, D. R., Altschule, M. D., and Volk, M. C., The effects on the cardiovascular system of fluids administered intravenously in man. I. Studies of the amount and duration of changes in blood volume. *J. Clin. Invest.*, 1938, 17, 7.
9. Gamble, J. L., Ross, G. S., and Tisdall, F. F., The metabolism of fixed base during fasting. *J. Biol. Chem.*, 1923, 57, 633.
10. Gamble, J. L., and McIver, M. A., Body fluid changes due to continued loss of the external secretion of the pancreas. *J. exper. Med.*, 1928, 48, 859.
11. Gamble, J. L., Extracellular fluid and its vicissitudes. *Bull. Johns Hopkins Hosp.*, 1937, 61, 151.
12. Peters, J. P., *Body Water: The Exchange of Fluids in Man*. Charles C. Thomas, Baltimore, 1935.
13. Peters, J. P., The distribution and movement of water and solutes in the human body. *Yale J. Biol. Med.*, 1933, 5, 431.
14. Gatch, W. D., and Little, W. D., Amounts of blood lost during some of the more common operations. *J. A. M. A.*, 1924, 83, 1075.
15. Butler, A. H., and Tuthill, E., An application of the uranyl zinc method for determination of sodium in biological material. *J. Biol. Chem.*, 1931, 93, 171.
16. Folin, O., *Laboratory Manual of Biological Chemistry*. D. Appleton-Century Co., New York, 1934, Fifth ed.
17. Wilson, D. W., and Ball, E. G., A study of the estimation of chloride in blood and serum. *J. Biol. Chem.*, 1928, 79, 221.

18. Van Slyke, D. D., and Sendroy, J., Jr., Carbon dioxide factors for manometric blood gas apparatus. *J. biol. Chem.*, 1927, 73, 127.
19. Wong, S. Y., The use of persulfate in the estimation of nitrogen by the Arnold-Gunning modification of Kjeldahl's method. *J. biol. Chem.*, 1923, 55, 427.
20. Howe, P. E., The use of sodium sulphate as the globulin precipitant in the determination of proteins in blood. *J. biol. Chem.*, 1921, 49, 93.
21. Wells, H. S., Youmans, J. B., and Mills, D. G., A formula and monogram for the estimation of the osmotic pressure of colloids from the albumin and total protein concentrations of human blood sera. *J. Clin. Invest.*, 1933, 12, 1103.
22. Van Slyke, D. D., and Neill, J. M., The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. *J. biol. Chem.*, 1924, 61, 523.
23. Gregersen, M. I., Gibson, J. J., and Stead, E. A., Plasma volume determination with dyes: errors in colorimetry; the use of the blue dye T-1824. *Am. J. Physiol. (Proc.)*, 1935, 113, 54.
24. Stewart, J. D., and Rourke, G. M., On the measurement of extracellular fluid volume with thiocyanate, and body fluid analyses in 33 normal individuals. *J. Lab. and Clin. Med.*, 1941, 26, 1383.
25. Stewart, J. D., and Rourke, G. M., Changes in blood and interstitial fluid resulting from surgical operation and ether anesthesia. *J. Clin. Invest.*, 1938, 17, 413.
26. Laviates, P. H., Bourdillon, J., and Klinghoffer, K. A., The volume of the extracellular fluids of the body. *J. Clin. Invest.*, 1936, 15, 261.

INTRAVENOUS MAGNESIUM SULFATE IN THE TREATMENT OF NEPHRITIC CONVULSIONS IN ADULTS^{1,2}

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Since 1926 the intravenous injection of magnesium sulfate has been employed in this clinic for the treatment of the twitchings and convulsions of advanced chronic nephritis in adults. This report is based on over forty cases so treated. Cases of eclampsia, acute nephritis of childhood, and various other vascular disorders also treated with intravenous magnesium sulfate have been included. For comparative purposes the physiological effects of intravenous magnesium were observed in subjects without cardiovascular disease.

REVIEW OF LITERATURE

On the basis of animal experiments Meltzer (1) recommended the use of magnesium intravenously in the treatment of tetanus, and there have been several reports of cases treated in this way (2, 3). In 1916 Peck and Meltzer described three cases in which magnesium sulfate was employed intravenously as a general surgical anesthetic (4). However, amounts sufficient to induce anesthesia were found to produce marked respiratory depression, so intravenous magnesium proved impractical as a general anesthetic. Attempts have been made to use it in combination with other anesthetics, but such suggestions have met with little favor (5).

In 1923 Blackfan (6), on the basis of Meltzer's work, introduced intravenous magnesium sulfate therapy in the treatment of the convulsions of acute nephritis in children. He was convinced of the efficacy of the treatment on purely clinical grounds, but was uncertain as to the exact mode of action. Originally, Blackfan was impressed with the importance of cerebral edema in the etiology of convulsions in his patients and combined intravenous magnesium sulfate therapy with dehydration produced by magnesium sulfate catharsis. Since magnesium sulfate intravenously injected acts through the specific pharmacological action of the magnesium ion, while its effects

are simply those of any saline cathartic when it is given orally, it is clear that two quite different modes of treatment were used in conjunction by Blackfan. In his earlier reports he is not clear as to the relative importance of the specific magnesium ion effects and of the non-specific dehydration effects. If cerebral edema were as important a factor in these cases as he originally believed, then dehydration should be most effective, and specific nervous system depression by magnesium should be harmful rather than otherwise; this, however, did not seem to be the case. In his last report in 1931 (7) he is inclined to stress the importance of dehydration but recognizes that intravenous magnesium sulfate may be quite effective without any dehydrating measures. Whatever the mode of action, Blackfan's work demonstrates that in acute nephritis of children the intravenous injection of magnesium sulfate in isotonic or hypotonic solution lowers blood pressure, stops convulsions, and in many instances is followed by immediate clinical improvement. It is remarkable that these effects are produced by amounts of magnesium sulfate far smaller than those required in Meltzer's experiments to produce anesthesia or to check tetanic spasms. Rubin and Rapoport (8) have also reported favorably on the use of intravenous magnesium sulfate in children but have emphasized the occasional dangers.

In 1925 and 1926 Lazard (9), Alton and Lincoln (10), and Lazard, Irwin and Vruwink (11) reported favorably upon the use of intravenous magnesium sulfate in controlling the convulsions of eclampsia, and it has since been used quite extensively for this purpose (12, 13). Its use, however, has been empirical, and its mode of action but little studied.

MATERIAL AND METHODS

Adult patients ordinarily received intravenously 500 cc. of a 2 per cent solution of the hydrated salt, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, during a thirty- to sixty-minute period. Sometimes smaller or larger amounts were given. The rate of injection was occasionally altered during the course of the infusion. The injection of 500 cc. was repeated after a few hours if convulsions recurred or if twitchings suggested that another convulsion might be impending. In a few instances a third injection was given. Over twenty of these cases have been observed by one of us (A. W. W.) during the course of the infusions. In the remainder it has been necessary to rely upon observations recorded

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² A preliminary report was presented to the American Society for Clinical Investigation in May 1940; *J. Clin. Invest. (Proc.)*, 1940, 19, 783.

in the chart; only those cases are included in which notes were made by observers of known competence.³

In most patients blood pressure was followed during and after the infusion. In certain cases serial electrocardiograms from lead two were taken. Concentrations of magnesium in serum, urine and feces were determined in a number of instances, using chemical methods previously described (14). Cerebrospinal fluid pressure was followed during the infusion in one case.

RESULTS

(A) Cardiovascular effects

Flushing, perspiration and a subjective sensation of extreme warmth were reported by all the conscious patients. This was usually first noted within less than half a minute after the commencement of the injection, and continued to a greater or less degree throughout the infusion. The face, neck and hands were particularly affected, and a rise in skin temperature was easily demonstrated. These evidences of cutaneous vasodilatation were as marked in subjects with acute and chronic vascular disease as in normal subjects. The intensity of the effect depended in part on the rate of injection.

Blood pressure was essentially unchanged during the infusion in four control subjects without cardiovascular disease, while in three others it dropped sharply (Figure 1). The drop, when it occurred, was usually quite sudden. Blood pressure varied but little during the earlier stages of the infusion; then quite suddenly both systolic and diastolic pressures broke sharply, and within one or two minutes might drop so low that they were difficult to measure (Figure 2). The fall in blood pressure sometimes continued for one or two minutes after the infusion had stopped. Within the next five or ten minutes the pressure then returned promptly to the normal range. This sharp collapse in blood pressure was regularly accom-

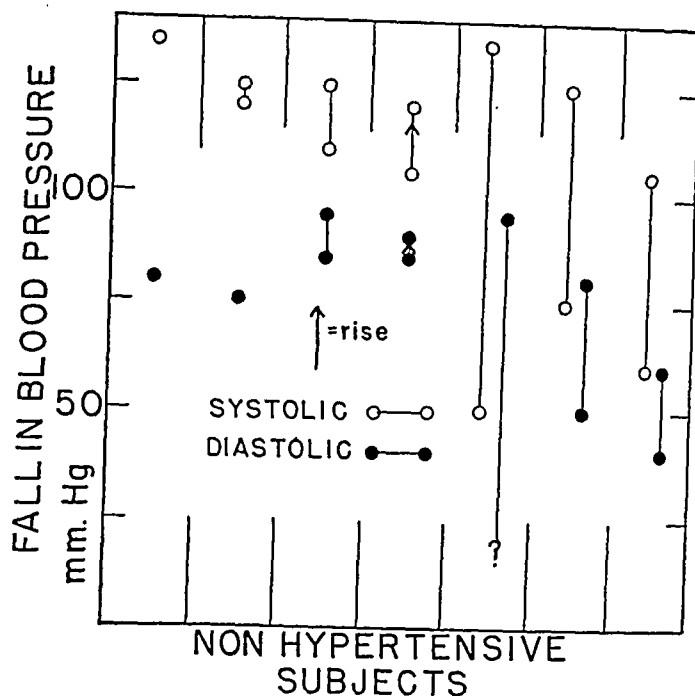


FIG. 1. EFFECT OF INTRAVENOUS MAGNESIUM SULFATE ON THE BLOOD PRESSURE OF SEVEN SUBJECTS WITHOUT HYPERTENSION OR VASCULAR DISEASE

The upper circle in each case represents the initial blood pressure; the lower, the minimum pressure reached during or after the injection. In the first four subjects there was no significant fall in blood pressure, while in the last three there was sudden collapse. One unresponsive patient received 250 cc., the other three 500 cc. each. The patients exhibiting decline of blood pressure received 220, 370 and 375 cc., respectively, of 2 per cent magnesium sulfate.

panied by nausea, thirst, extreme restlessness, mental confusion and a subjective sense of imminent doom. These alarming symptoms disappeared at once when the pressure rose again after stopping the infusion. So disturbing were these manifestations that at the height of the reaction adrenalin was administered to one subject and intravenous calcium chloride to another. Since recovery was as rapid in the third untreated case as in these two, these procedures cannot be held solely responsible for the rapid return of the blood pressure to normal. The pulse rate was always slow during the period of hypotension. The four subjects exhibiting no significant fall in blood pressure complained of no such violent subjective disturbances but only of a feeling of warmth.

The response of the blood pressure in patients with cardiovascular and renal disease to the intravenous injection of magnesium sulfate in certain respects resembled, and in others differed from,

³ For help in this connection we are indebted to the entire past and present staff of the Metabolism Division of the Department of Medicine. Particular use has been made of the meticulous observations of certain cases by the late Dr. Maurice Wakeman, who originally introduced the use of intravenous magnesium sulfate in this clinic in 1926. Dr. John P. Peters has had the responsibility for directing the treatment since its introduction and has made available the records of cases not studied by us personally. Acknowledgment is made to Dr. Arthur H. Morse and to Dr. Grover F. Powers for permission to include the obstetrical and the pediatric cases.

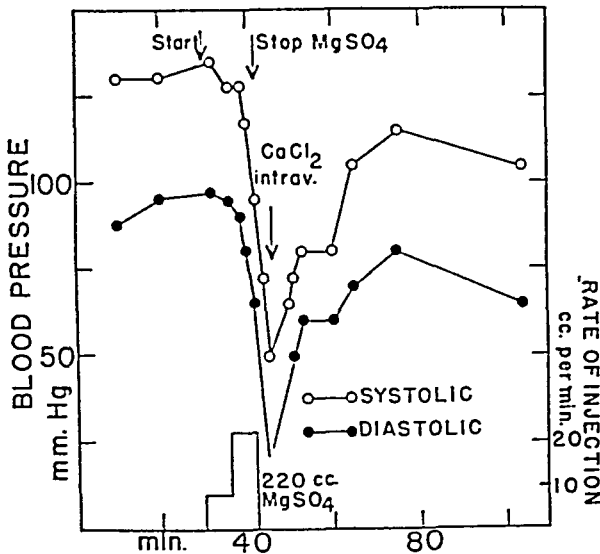


FIG. 2. SUDDEN COLLAPSE OF BLOOD PRESSURE IN A NORMAL SUBJECT WHILE RECEIVING MAGNESIUM SULFATE INTRAVENOUSLY

Just after the termination of the injection the patient was unconscious and the diastolic pressure was unobtainable. CaCl_2 was given intravenously at this time. Recovery was complete within a few minutes.

that of subjects without vascular disease. As with normal subjects, the blood pressure was but little affected in one group of cases (Figure 4), while it fell markedly in another group (Figure 3). The different types of chronic renal and cardiovascular disease were evenly distributed between the two groups which were indistinguishable on the basis of age and sex distribution or on the basis of amount or rate of injection of magnesium. In occasional cases the blood pressure reacted at one time and failed to react at another during the course of the same illness. In contrast to this irresponsive behavior of many of these chronic patients, the blood pressure fell promptly in all but one of the subjects with eclampsia and acute nephritis (Figure 5).

In one important respect the reaction of hypertensive subjects differed from that of those without cardiovascular disease. The sharp collapse of the blood pressure in normal subjects receiving intravenous magnesium was rarely observed in those with hypertension. On the contrary, the blood pressure usually fell rather slowly and gradu-

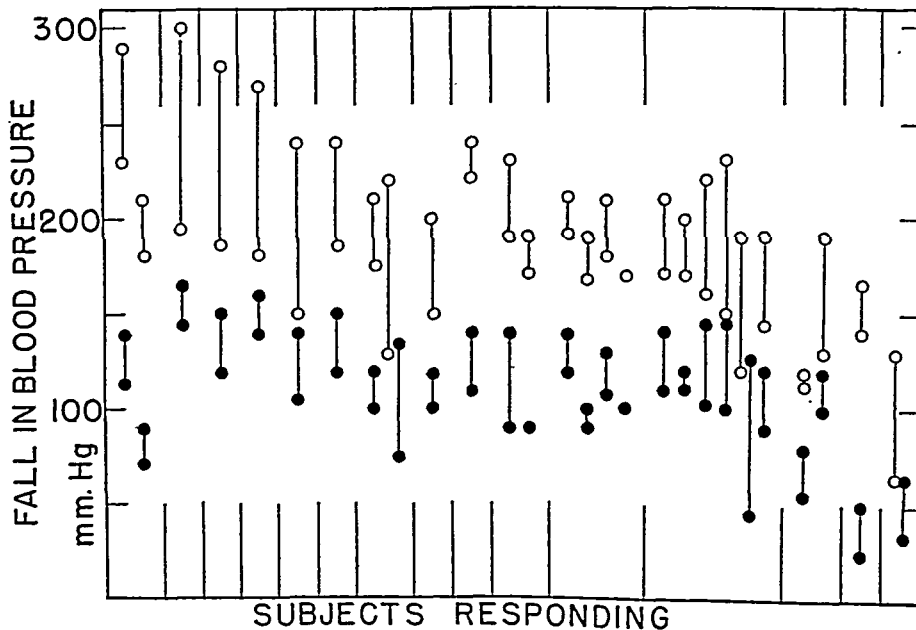


FIG. 3. CHANGE IN BLOOD PRESSURE FOLLOWING INTRAVENOUS MAGNESIUM SULFATE IN THE FIFTEEN SUBJECTS WITH CHRONIC RENAL AND VASCULAR DISEASE WHOSE PRESSURE DID DECLINE

The symbols are the same as those used in Figure 1. Multiple injections in the same patients are indicated by the bracketing lines at the top and bottom. The usual dose was 500 cc. in each injection.

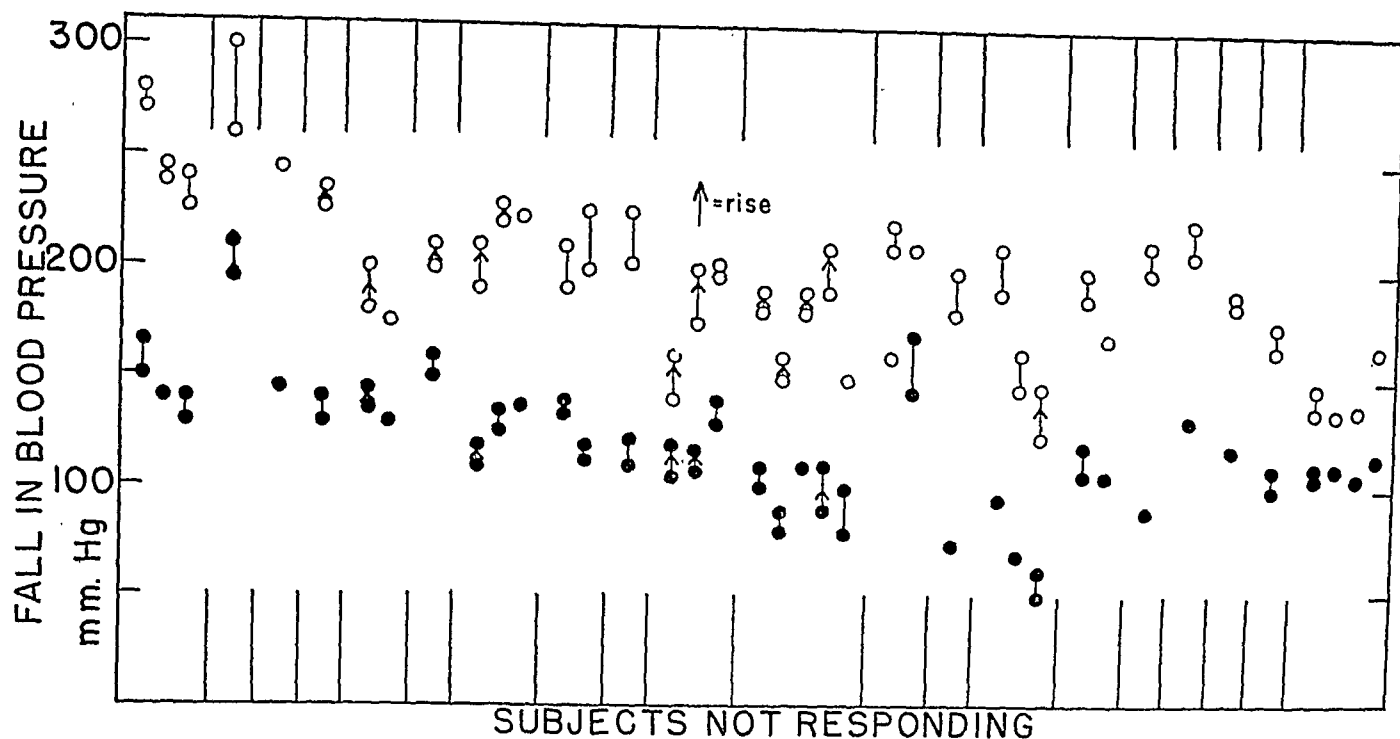


FIG. 4. CHANGE IN BLOOD PRESSURE FOLLOWING INTRAVENOUS MAGNESIUM SULFATE IN THE TWENTY SUBJECTS WITH CHRONIC RENAL AND VASCULAR DISEASE WHOSE PRESSURE DID NOT DECLINE SIGNIFICANTLY. The symbols are identical with those used in Figures 1 and 3. The usual dose was 500 cc.

ally (Figure 6) without any distressing subjective symptoms. It was hardly ever necessary to discontinue the infusion because of symptoms associated with fall in blood pressure, and the shock-like levels reached in normal subjects were seldom seen. The degree to which blood pressure fell was

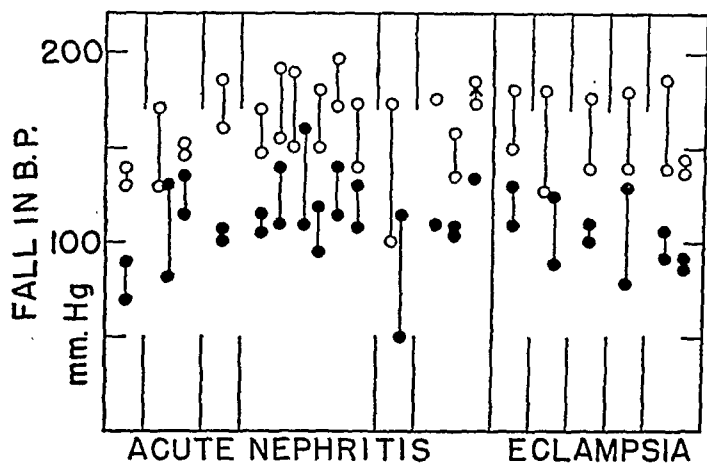


FIG. 5. CHANGE OF BLOOD PRESSURE FOLLOWING INTRAVENOUS MAGNESIUM SULFATE IN SIX SUBJECTS WITH ACUTE NEPHRITIS AND IN FIVE SUBJECTS WITH ECLAMPSIA.

The symbols are identical with those used in previous figures. The subjects with acute nephritis were children, the dose varying between 100 cc. and 300 cc. The eclampsia patients usually received 500 cc.

not closely correlated with the amount of magnesium which had been given but rather with the rate of administration. It is not unlikely that some of the "unresponsive" subjects, with or without cardiovascular disease, might have developed hypotension if the rate of infusion had been greatly accelerated. The fall in blood pressure, when it occurred, was apt to be somewhat more persistent in the hypertensive than in the normal subjects, frequently lasting half an hour to an hour and occasionally lasting several hours. The return to the previous level took place in spite of the continued presence of an increased concentration of magnesium in the serum (Table II).

No correlation could be found between cessation of convulsions and the presence or absence of blood pressure changes. Electrocardiograms were taken during the entire course of magnesium injection in six instances. Minor changes only were observed, consisting chiefly of slowing and slight prolongation of the PR interval. No intraventricular block and no arrhythmia were detected. The only case in which the prolongation of the PR interval was at all evident was also the one in which the serum magnesium reached the highest concentration found in this series.

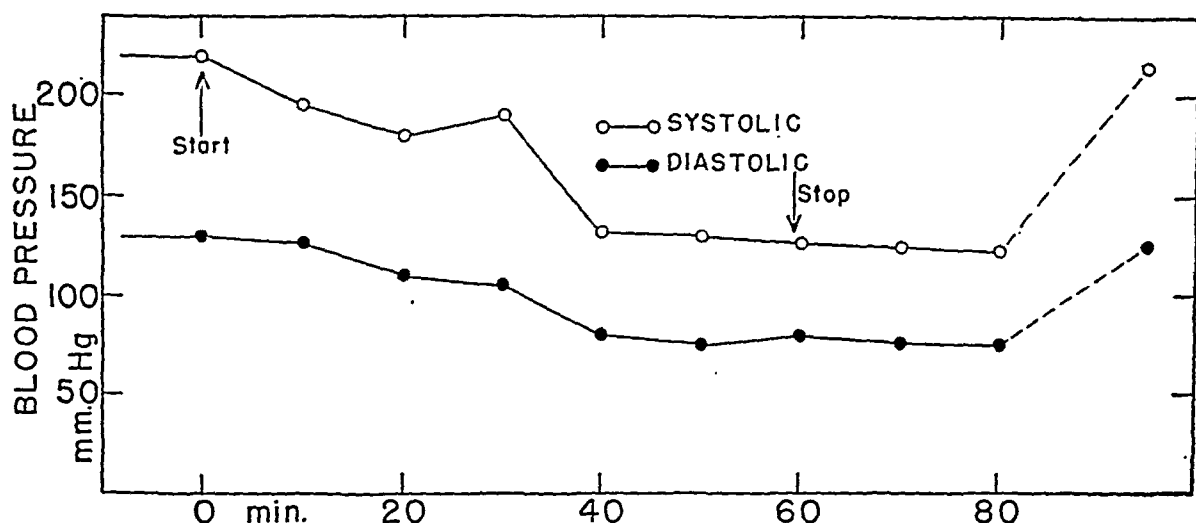


FIG. 6. GRADUAL DECLINE OF BLOOD PRESSURE DURING THE INTRAVENOUS INJECTION OF 500 CC. OF MAGNESIUM SULFATE IN A PATIENT WITH CHRONIC GLOMERULONEPHRITIS AND SEVERE HYPERTENSIVE CARDIOVASCULAR DISEASE

(B) Effects on convulsions and twitchings

It is difficult to evaluate the effect of any therapeutic measure on the convulsions in "uremic" patients for several reasons. First, only a portion of the patients with pre-terminal nitrogen retention and manifestations of "uremia" develop convulsions. Although twitching of the muscles—sometimes fibrillary, sometimes involving whole muscles and groups of muscles—is often a prelude to the development of actual convulsions, the appearance of convulsions is by no means invariable. Secondly, therapy instituted after the development of convulsions cannot readily be evaluated since convulsions tend to disappear spontaneously. Finally, the great majority of the patients studied here were near death when therapy was instituted, since convulsions and twitching in chronic nephritis with nitrogen retention seldom occur until shortly before death. Failure of convulsions to recur after a single injection in many cases was therefore inevitable. Nevertheless it was possible to make an analysis of the relation of the convulsions to therapy. This is presented in Table I.

Some two-thirds of the cases of chronic nephritis with convulsions had no more convulsions after a single injection of magnesium sulfate, while the remaining third required two injections. No patient who received full amounts in each injection required more than two injections. The precise

interpretation of this apparently effective control of convulsions is uncertain, since it is impossible to say how many patients in an untreated group

TABLE I
Effectiveness of intravenous magnesium sulfate in relieving convulsions and muscular twitching

Cases receiving magnesium for other causes are not included.	
Group A: Chronic nephritis with convulsions (25 cases)	
Complete cessation of convulsions after one injection *	17
Complete cessation of convulsions after two injections	7
Complete cessation of convulsions after three injections †	1
In only one of these 25 cases had the patient been given magnesium therapy for twitching prior to the development of convulsions.	
Group B: Chronic nephritis without convulsions but with twitching (9 cases)	
"Believed helpful"	5
"Believed useless"	3
Uncertain	1
In none of these nine cases did actual convulsions develop.	
Group C: Acute nephritis with convulsions and subsequent recovery in children (2 cases)	
Convulsions ceased after one injection	1
Single recurrence after one injection	1
Group D: Eclampsia and convulsions (8 cases)	
Convulsions ceased completely after one injection ‡	7
Convulsions ceased completely after two injections	1

* In three instances convulsions recurred after a free interval of a week or more.

† Convulsions continued during the third injection. The first two injections were half the usual size.

‡ Of these one died during the infusion and one died twelve hours after the infusion.

of cases would have had more than two sets of convulsions. It is our general clinical impression that, while convulsions are often single in uremia, cases are not infrequently met with in which repeated sets of convulsions occur. If this be so, there are grounds for believing that magnesium therapy is effective, since in every instance in our treated series convulsions were readily controlled.

More definite is the fact that, considering groups A and B together, in only one instance out of the ten in which preliminary twitchings had been treated with magnesium did convulsions develop. Since twitchings are ordinarily so frequently followed by convulsions, it seems almost certain that this represents a genuine prophylactic action of magnesium. The effects of magnesium in stopping the twitching itself are very slight. In no instance was magnesium followed immediately by disappearance of the twitching.

Magnesium is apparently effective in controlling the convulsions in acute nephritis and in eclampsia (Table I, groups C and D). It is difficult to interpret these figures, however, since convulsions are somewhat less apt to be persistently recurrent in cases of this type, and the total number of cases studied is small.

(C) *General clinical improvement*

Notes stating that there was a "general clinical improvement" are frequently found in connection with the eclampsia and acute nephritis cases and are notably absent in the cases with chronic nephritis. This is such a vague expression that extended discussion is out of place in an attempt at objective evaluation of the results of therapy. Diuresis was but little stimulated; vomiting and mental status were not often affected. A deep natural sleep sometimes occurred not long after the infusion.

(D) *Effects on cerebrospinal fluid pressure*

It is well known that, whatever may be the case with the acute nephritis of children, the brains of adult uremic patients do not regularly show edema at autopsy (15). Lumbar puncture frequently reveals a slightly increased pressure during life, but rarely a markedly increased pressure (16). Of the seven instances in this series in which lumbar punctures were done, a significantly in-

creased pressure was found in only one, a patient who proved at autopsy to have a meningioma. In view, however, of Blackfan's suggestion that intravenous magnesium sulfate might in some manner achieve its effects by lowering cerebrospinal fluid pressure, a lumbar puncture needle attached to a manometer was inserted in the spinal canal just before an infusion of magnesium sulfate. The patient was a stuporous young woman in the last stages of glomerulonephritis who had been having convulsions shortly before the infusion. Before the infusion the cerebrospinal fluid pressure was 260 mm. of water, during the half hour infusion it was 230 mm., and for some time thereafter it remained at this same level. Evidently magnesium sulfate had no significant effect on the cerebrospinal fluid pressure in this case.

(E) *Untoward reactions*

Some 100 or so injections were given to fifty-three patients, most of whom were in a pre-mortal state with advanced renal and cardiac disease. Nevertheless death only occurred once during an infusion, and this was just after the infusion had begun. This patient was desperately ill with an acute eclamptic syndrome, and it is probable that she would have died within an hour or so even if no magnesium sulfate had been given. With this single exception no serious ill effects which might have been ascribed to the magnesium were noted.⁴ The sudden fall of blood pressure occasionally appeared alarming but was never followed by any persistent shock-like state. Even after hypoten-

⁴ Through the courtesy of Dr. Charles Aring we have been informed of a fatality immediately following the rapid intravenous injection of 30 cc. of 25 per cent magnesium sulfate. This patient received in less than a minute as much magnesium as that contained in 375 cc. of our 2 per cent solution. Such a rapid injection produces a sudden great elevation of magnesium in the serum followed by a rapid fall. The maximal concentration attained in serum under these circumstances depends as much upon rate of injection as upon the amount injected, so that it is difficult to control it with any precision. In view of the narrow margin between effective and dangerous concentrations, this is a serious objection. The occurrence of this fatality emphasizes the dangers inherent in the rapid intravenous injection of concentrated solutions of magnesium sulfate. In our therapeutic practice we use no greater concentration than 2 per cent, and allow at least half an hour for the injections of 500 cc.

sion had been established by the infusion of magnesium sulfate, a convulsion invariably raised blood pressure, indicating that vasoconstrictor mechanisms were still effective and that blood pressure was not fixed at the low level. Amounts sufficient to cause failure of spontaneous respiration were never used. Vomiting during the infusion occurred twice.

(F) *Concentrations in the blood, and excretion*

In Table II are presented analyses of serum for magnesium before and after infusion in nine

subjects. In four of them urinary excretion, and in one excretion in the stools, was studied as well. The serum concentration immediately following the infusion usually ranged from 4 to 10 m.eq. per liter, although in two instances a concentration of 13.3 m.eq. per liter was found. These high values were associated with slight shallowness and slowing of respiration but no respiratory failure. Knee jerks, however, were absent. Patient Number five was comatose before and during the infusion; but patient Number eight was awake and cooperative. Toward the

TABLE II
Magnesium in serum, urine and stools

Number	Diagnosis	Date	Body weight	Blood NPN	Magnesium				
					Injected	Serum			Urine 24 hours after infusion
						Initial	After infusion	Next morning	
			kgm.	mgm. per cent	m.eq.	m.eq. per liter			m.eq.
(1)	Chronic nephritis with edema	December 12	60	250	81.3	4.9	8.4*	7.5	
(2)	Eclampsia	February 2	50	40	122.1	2.7	5.8*		
(3)	Chronic nephritis with edema	November 15	50	185	37.7	2.4	4.3	2.6	
		November 17				2.0			1.0
		November 18 to November 21							4.4†
		November 27		200	73.2	2.0	5.4	4.5	1.4
		November 27 to December 5							3.0†
(4)	Chronic pyelonephritis	February 10	60	113	81.3	1.6	6.6	4.3	
		February 19		129	81.3	2.6	7.1	5.9	
		February 23		173	81.3	4.8	8.9	8.0	
(5)	Chronic nephritis with edema	May 16	52	84	122.0	1.7	7.7	4.4	9.2
		June 21		150	97.6	6.7‡	13.3	12.2	26
(6)	Chronic nephritis without edema	October 21	55	145	81.3	1.8	6.4		30
(7)	Chronic nephritis with slight edema	December 16	60	200	81.3	4.3	10.8		19
		December 16 to December 18						7.0§	7.5
(8)	Acute necrotizing hypertensive disease	December 29	50	50	81.3	2.3	9.8	4.9	
		December 30			81.3	?	11.0	2.8	
		December 31		24	81.3	2.8	13.3	3.0	83.0
		January 5			81.3	2.5	8.5	2.4	
		January 6			61.0	2.4	8.4	1.9§	
		January 8			81.3	1.9	10.5	2.4§	
		January 10			81.3	2.4	7.7		
(9)	Chronic nephritis without edema	March 4	55	50	81.3	1.5	4.4	2.5	
(10)	Chronic nephritis without edema	May 29	50	45	81.3	2.3	6.7		
		May 30			81.3		8.2		
		May 31			81.3		8.5		

* Six hours after infusion.

† Stool magnesium 2.6 and 4.5 m.eq. per 24 hours during these two periods, respectively.

‡ 64.9 m.eq. of magnesium given during the previous night.

§ Forty-eight hours later.

end of the infusion associated with this 13.3 value, this second patient became very drowsy and responded only with difficulty; sensitivity to painful stimuli was also much reduced. These symptoms disappeared not long after the end of the infusion. Levels of 4 to 10 m.eq. per liter were not accompanied by anesthesia, coma, motor paralyses, absence of deep reflexes, or any subjective sensation other than the feeling of warmth during the injection. This confirms the observations of Peck and Meltzer (4), who found that amounts sufficient to raise the concentration to about 15 m.eq. per liter (estimated) were necessary to produce surgical anesthesia in man. It is also consistent with the observations of Neuwirth and Wallace (17) and of Hoff, Smith and Winkler (18) that levels in excess of 10 m.eq. per liter are necessary to produce neuromuscular block and evidence of narcosis in dogs.

The rate of decline of the concentration of magnesium in the serum of subjects with high blood NPN values is very slow. Thus in the first five cases of Table II the magnesium concentration had usually dropped only a milliequivalent or so by the next morning. This rate of disappearance means that an elevated concentration must persist for several days. On the other hand, the rate of disappearance from the serum was very rapid in those patients whose renal function was more nearly normal (patients Number eight and Number nine), so that the concentration had virtually returned to normal by the next day. The urinary excretion is correspondingly low in the patients with markedly impaired renal function. Thus only 8 per cent of the injected magnesium was recovered from the urine in the twenty-four hours following the infusion in patient Number five. In Case eight, on the other hand, with a normal NPN, the entire amount injected on December 31 was recovered from the urine the next day. Stool excretion was not increased following magnesium injection (patient Number three), so it must be assumed that the main channel of excretion of injected magnesium is the urine, and that in advance nephritis with depressed clearances this excretion is ordinarily much impaired. The importance of renal excretion in the elimination of injected magnesium is consistent with the results of McCance and Widdowson with normal men (19)

and of Smith, Winkler and Schwartz with dogs (14).

In the last column of Table II are tabulated the apparent volumes of distribution of the injected magnesium in cases with renal insufficiency. These values were calculated by the formula (14):

Volume of distribution (per cent of body weight)

$$= \frac{\text{Amount of Mg injected}}{\text{Increase in concentration of Mg in serum}} \times \frac{100 \times 0.93}{\text{Body weight}}$$

This calculation ignores the urinary excretion during the period of injection, an omission which is only justified by the low rate of excretion of magnesium in patients with renal insufficiency. It cannot be applied to the cases with normal or slightly reduced renal function. The factor 0.93 is assumed to be the fraction of water in serum. The results indicate that the immediate apparent volume of distribution varies between 19 and 40 per cent of the body weight and tends to be higher in edematous than in non-edematous subjects. These figures are consistent with those obtained in normal animals and indicate that the greater part of the injected magnesium is, at least at first, confined to the extracellular fluid (14).

DISCUSSION

Intravenous magnesium in the amounts given is apparently of some value in preventing and controlling the development of convulsions in chronic nephritis. These benefits, such as they are, occur with amounts of magnesium so small that in animals comparable amounts produce no demonstrable depression of the nervous system (17, 18). They do, however, produce pronounced vasodilatation (20), and in man the same vasodilatation is regularly observed. It seems at first sight reasonable, therefore, to ascribe the therapeutic effectiveness of the ion more to this vasodilatation than to nervous system depression. This interpretation is, however, not consistent with the observation that in many of the cases of chronic nephritis no fall in blood pressure occurred. It is precisely in the chronic group that beneficial effects were also so irregular, in contrast to the much more consistent fall in blood pressure with clinical improve-

ment in the subjects with acute nephritis and eclampsia. That some moderate peripheral or central depression of the nervous system occurred as well cannot, of course, be excluded. With repeated injections of magnesium (Table II (5, 8)), the concentrations of magnesium rose above 10 m.eq. per liter, and with such concentrations depressions of the nervous system are demonstrable in dogs (17, 18). Slow single injections of 500 cc. of 2 per cent MgSO_4 did not raise the serum magnesium to this extent.

The slow excretion of magnesium in the urine of nephritic patients caused a persistent elevation of magnesium in the serum, lasting some hours or days. This makes repeated doses more effective and at the same time more dangerous. Since 1000 cc. of 2 per cent MgSO_4 will raise the magnesium concentration to the 10 to 15 m.eq. per liter level, and since in animals respiratory failure occurs at 15 m.eq. per liter, not more than two injections of 500 cc. each should be given within a period of two or three days. The situation with patients with eclampsia is not comparable, since renal function is usually more nearly normal and rapid elimination of the magnesium from the blood stream is to be expected.

The concentrations of magnesium attained were insufficient to cause respiratory arrest in any instance, but in several patients mild depression of respiration was observed. In all such cases tendon reflexes had already disappeared. The results obtained with animal experiments (18) were thus confirmed, in that knee jerks disappear at a concentration of magnesium below that at which respiration fails. Practical advantage may be taken of this fact in regulating the amount and rate of injection of magnesium in man. So long as the knee jerks are active, there is no need to be concerned about respiratory failure, while their disappearance is a warning sign.

The return of the blood pressure to its previous level in the continued presence of an elevated concentration of magnesium in serum may only mean that the concentration, though still greater than normal, has fallen below the critical level necessary to overcome vasoconstrictor impulses. During intravenous injection the rate of diffusion of magnesium out of the blood stream into the tissues may well be somewhat less than the rate of injection, so that the concentration rises sharply in the

serum. So long as this is in progress a concentration greater than that in tissues and sufficient to lower the blood pressure may be temporarily maintained in the circulating serum. After the infusion is concluded, magnesium in serum will gradually decline until equilibrium with tissue fluids is reached, even if excretion is negligible. The greater persistence of hypotension in subjects with renal impairment than in those without may in part be due to the more rapid fall of magnesium of serum in the latter, due to urinary excretion as well as diffusion. It is also possible that powerful vasoconstrictor forces may be called into play in order to antagonize the action of magnesium. The nature of these hypothetical stimuli is as obscure as the locus of action of magnesium itself. Even after a marked hypotension has been induced by magnesium, any muscular activity of the patient raises the blood pressure sharply. From the ease and rapidity with which the fall of blood pressure may be reversed, it may reasonably be concluded that there is no capillary paralysis such as is seen in "shock." It seems reasonable to believe that the main site of its action is the arteriole. In this event it must be assumed that in the unresponsive subjects the cutaneous vessels dilate but that the splanchnic or somatic vessels contract, thus maintaining the blood pressure.

The effects on the cardiovascular system are not entirely comparable to those obtained experimentally in normal dogs (20, 21). Both cutaneous vasodilatation and fall in blood pressure are present in the dog. These follow the injection of amounts too small to exert any demonstrable effect on the nervous system. The fall in pressure, however, is apt to be gradual in the dog and the sudden collapse seen in normal man was not observed. Indeed, the normal dogs behaved more like the hypertensive patients. The action in dogs is usually transient, but repeated doses are effective each time and there is a tendency for the blood pressure to remain at a somewhat reduced level as long as an elevation of magnesium in the serum persists. This vasodilator effect occurs with as little as 3 to 5 m.eq. of magnesium per liter of serum, whereas motor paralysis and respiratory arrest do not occur until a level of 10 to 15 m.eq. is attained. Significant electrocardiographic changes also occur during magnesium injection in the dog, consisting chiefly of progressive pro-

longation of the PR interval and, finally, bradycardia and cardiac arrest (21). These changes are, however, barely manifest at levels below 15 m.eq. per liter and can often be demonstrated only with the aid of artificial respiration. The slight changes observed in man are entirely comparable with those produced in the dog by similar concentrations of magnesium in the serum.

CONCLUSIONS

1. The slow intravenous injection of 500 cc. of 2 per cent magnesium sulfate ($\text{MgSO}_4 \times 7 \text{H}_2\text{O}$) is a safe procedure which is of some value in preventing and controlling the convulsive seizures of chronic nephritis in adults. The benefits are, however, neither certain nor dramatic.

2. If convulsions are not controlled by a single injection, they will almost always be checked by a second injection. After two injections magnesium may attain such a high concentration in serum that its depressant effects on the nervous system may become manifest.

3. After injection the concentration of magnesium may remain elevated for several days in patients with chronic nephritis because of the retarded excretion of magnesium in the urine. More than 1000 cc. of 2 per cent magnesium sulfate should therefore not be given within a forty-eight-hour period in the presence of severe renal insufficiency, lest a concentration sufficient to produce respiratory paralysis be attained.

4. Magnesium in these amounts causes cutaneous vasodilatation in all subjects. Blood pressure regularly falls in subjects with acute nephritis or eclampsia but is frequently unaffected in those with chronic cardiovascular disease.

BIBLIOGRAPHY

1. Meltzer, S. J., Inhibitory properties of magnesium sulfate and their therapeutic application in tetanus. *J. A. M. A.*, 1916, 66, 931.
2. Blake, J. A., The use of magnesium sulfate in the production of anesthesia and in the treatment of tetanus. *Surg. Gynec. and Obst.*, 1906, 2, 541.
3. Meakins, J. C., *The Practice of Medicine*. Second edition, C. V. Mosby Co., St. Louis, 1938, p. 1239.
4. Peck, C. H., and Meltzer, S. J., Anesthesia in human beings by intravenous injection of magnesium sulfate. *J. A. M. A.*, 1916, 67, 1131.
5. Beckman, H., The alleged synergism of magnesium sulphate and morphine. *J. A. M. A.*, 1925, 85, 332.
6. Blackfan, K. D., and Hamilton, B., Uremia in acute glomerular nephritis. Boston M. and S. J., 1925, 193, 617.
7. Blackfan, K. D., and McKhann, C. F., Acute glomerular nephritis in children. Treatment of cerebral manifestations. *J. A. M. A.*, 1931, 97, 1052.
8. Rubin, M. I., and Rapoport, M., The mode of action of magnesium sulfate in reducing the hypertension of acute glomerulonephritis. *Am. J. M. Sc.*, 1941, 201, 734.
9. Lazard, E. M., A preliminary report on the intravenous use of magnesium sulphate in puerperal eclampsia. *Am. J. Obst. and Gynec.*, 1925, 9, 178.
10. Alton, B. H., and Lincoln, G. C., The control of eclampsia convulsions by intraspinal injections of magnesium sulphate. *Am. J. Obst. and Gynec.*, 1925, 9, 167.
11. Lazard, E. M., Irwin, J. C., and Vruwink, J., The intravenous magnesium sulphate treatment of eclampsia. A collective report of 142 cases. *Am. J. Obst. and Gynec.*, 1926, 12, 104.
12. Stroganoff, W., and Davidovitch, O., Two hundred cases of eclampsia treated with magnesium sulphate (MgSO_4): a preliminary report. *J. Obst. and Gynaec. Brit. Emp.*, 1937, 44, 289.
13. Lazard, E. M., An analysis of 575 cases of eclampsia and preeclamptic toxemia treated by intravenous injection of magnesium sulphate. *Am. J. Obst. and Gynec.*, 1933, 26, 647.
14. Smith, P. K., Winkler, A. W., and Schwartz, B. M., The distribution of magnesium following parenteral administration of magnesium sulfate. *J. Biol. Chem.*, 1939, 129, 51.
15. Foster, N. B., Uraemia. *Harvey Lectures*, 1920-1921, p. 52.
16. Merritt, H. H., and Fremont-Smith, F., *The Cerebrospinal Fluid*. W. B. Saunders, Phila., 1937.
17. Neuwirth, I., and Wallace, G. B., On the use of magnesium as an aid in anesthesia. *J. Pharmacol. and Exper. Therap.*, 1929, 35, 171.
18. Hoff, H. E., Smith, P. K., and Winkler, A. W., Effects of magnesium on the nervous system in relation to its concentration in serum. *Am. J. Physiol.*, 1940, 130, 292.
19. McCance, R. A., and Widdowson, E. M., The fate of calcium and magnesium after intravenous administration to normal persons. *Biochem. J.*, 1939, 33, 523.
20. Hoff, H. E., Smith, P. K., and Winkler, A. W., The relation of blood pressure and concentration in serum of potassium, calcium and magnesium. *Am. J. Physiol.*, 1939, 127, 722.
21. Smith, P. K., Winkler, A. W., and Hoff, H. E., Electrocardiographic changes and concentration of magnesium in serum following intravenous injection of magnesium salts. *Am. J. Physiol.*, 1939, 126, 720.

THE SEROLOGICAL TYPING OF HEMOLYTIC STREPTOCOCCI OF THE LANCEFIELD GROUP A

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The relationships among the hemolytic streptococci have been greatly clarified by the fundamental work of Lancefield (1). She has shown that these organisms may be classified into groups on the basis of a precipitin reaction and ample evidence has accumulated (2, 3) to show that almost all human infections are caused by members of her Group A.

Strains of the groups from B to K are responsible for many types of infection in animals, and occasionally in man, and are also frequent normal inhabitants of the human nose, throat and bowel. Non-hemolytic varieties are also well known among these groups (4).

The strains which are members of Group A may be further classified into more than 30 types by an agglutination test as described by Griffith (5). Many technical difficulties have prevented the widespread application of this procedure but the modifications of the original method suggested by Ward and Rudd (6) and further revised in this laboratory have made the serological typing of these streptococci simple and reliable.

The development of a satisfactory method of classifying the hemolytic streptococci pathogenic for man into types has offered a tool for the investigation of many aspects of the epidemiology, nature, and therapy of infections due to these organisms. Except for studies of epidemiology and observations on the relation of type to immunity, practically no answers have yet been obtained to the challenging questions which the availability of this method has raised.

A continuing study of hemolytic streptococcal infection in the San Francisco area has, therefore, been undertaken, and the Griffith classification has been applied for the purpose of gaining further information on the relationship of the serological types of Group A to disease states.

It is the purpose of this paper to review the published data bearing on this subject, to present

certain modifications of method which increase the simplicity and reliability of the serological typing of the hemolytic streptococci and to state and discuss the results of the application of these methods to strains of streptococci isolated from infections and from normal human subjects in San Francisco during the seasons of 1940 and 1941.

REVIEW OF THE LITERATURE

Distribution of Griffith types in various disease states. Table I presents the collected data on nearly all of the extensive surveys of Griffith type distribution in various disease states available at the present time. For purposes of simplification and clarity no type occurring in less than 2 per cent of the examined strains in any series has been included and all percentage values are given to the nearest whole number.

From a study of these data certain important facts emerge. It may be definitely stated that no type or small group of types is regularly responsible for scarlet fever throughout the world. Types 1, 2, 3, and 4 occur frequently in Great Britain, the United States and Japan, but even in these areas a very large number of cases may be caused by other types. For example, in Edinburgh in 1933 to 1934, 66 per cent, and in London in 1937, 53 per cent of all strains did not fall into these four types. In Australia, types not included in those originally described by Griffith and identified locally¹ are isolated from the majority of

¹ Since the preparation of this paper, an opportunity to study strains of the local Australian types has been made available through the courtesy of Dr. F. G. Morgan of the Department of Health, Commonwealth of Australia.

Type "Hempston" appears to be identical with the Griffith type 3; type "Wade" with type 9; type "Stewart" with type 13; and type "Coghlan" with type 23.

"Wade" occurred more frequently than any other local type and is included in a separate column in table I. Fifty-six and a half per cent of the strains included in the same table under "local surgical types" were of types "Hempston," "Stewart" and "Coghlan."

general tendency for the two groups of diseases to be caused by different groups of organisms. For example, in London, only 22 per cent of cases of puerperal sepsis were caused by hemolytic streptococci of the 5 types most commonly found in scarlet fever, and in Australia the common scarlet fever types only occasionally were responsible for surgical infections and puerperal sepsis.

Epidemiology. Enormously valuable contributions to the knowledge of the epidemiology of hemolytic streptococcal infections have been made by the introduction of the Griffith classification. Some of these have been discussed in the previous section.

Colebrook (11) has shown that, in puerperal infections, the source of the infections may be traced in a large number of instances. In approximately 10 per cent it is found that the infecting organism has been harbored in the nasopharynx of the infected individual, but practically never in her genital passages. In the remaining cases, a physician, nurse, attendant, or member of the family is found to be the responsible carrier.

The comprehensive studies on war wound infections by Miles (18) have demonstrated that infections due to the hemolytic streptococcus spread from patient to patient and that waves of new infections follow the introduction into the ward of a fresh case infected with a new type. Epidemics of streptococcal respiratory infections within a closed group have been shown to be caused by a single type of streptococcus (19).

Severity of infection and complications. In spite of the large number of observations recorded in Table I, little information has been collected bearing on the relationship of the Griffith types to the severity of infection nor to the nature and type of complications. It has been shown by Brown and Allison (20) that suppurative complications such as otitis media following scarlet fever are usually the result of reinfection with a type different from that causing the initial infection and these observations have been amply confirmed by de Waal (10). As a general rule, complications occurring in the first week are caused by the initial type; those developing after this time are due to new types. In de Waal's series of 1,831 cases, 600 patients had complications of which only 20 per cent were associated with the type present on admission.

An analysis of his data, for the purpose of determining the relative tendency of various types to produce a more severe disease or complications, shows fairly clearly that there is a very real difference in the ability of the strains of the various types to cause complications and to prepare the soil for infection by other types.

Thus 48 per cent of initial Type 4 infections suffered complications, 30 per cent of Type 1, 18 per cent of Type 2, and only 7 per cent of Type 3. Type 1, which was the etiological agent in 49 per cent of the admitted cases, caused only 22 per cent of complications, whereas Type 4, which was isolated from only 13 per cent of all cases on admission, was responsible for 20 per cent of all complications. The Type 4 cases are interesting for, not only were they more often complicated, but 66 per cent of the complications were caused by Type 4 rather than a new type.

Type 4 also appears to have caused otitis media about twice as frequently as Types 1 and 2. Organisms of this type were therefore more virulent and highly invasive than those of the other common types in this particular epidemic.

Other observations bearing on these points have been made by Bailey (13), who found no difference in the complication rate among the cases included in Types 2, 3, and 6, his most frequently occurring types, and Pauli and Coburn (14) who have stated that, during a certain season in New York, Type 13 infections were more likely to reactivate rheumatic fever than were infections due to Type 22.

It is impossible, therefore, to state definitely whether strains of certain types, when isolated from infections over wide geographical areas, will be uniformly more virulent than others, or whether this phenomenon will be a purely local one, varying from season to season, or epidemic to epidemic, but it is clear that there have been differences in the invasiveness of certain types in carefully studied epidemics.

Animal virulence and toxin formation. An extensive study of the relation of the serological type of Group A streptococci isolated from cases of scarlet fever to animal virulence has been made by Kodama and his associates (16c). A constant and very large infecting dose of organisms was used with white mice as test animals. Sixty-one

strains of Type 2, 189 of Type 4, and 77 of Type 8 were studied. The mortality rates in the infected animals were 16.4, 25.4, and 20.8 per cent, respectively. Too small a number of strains of other types were studied for satisfactory conclusions to be drawn. The differences in mortality are small and not statistically significant, but suggest that Type 4 was more virulent than Type 2. They further studied 10 strains each of 5 types after serial mouse passage, but failed to demonstrate any difference in the degree of mouse pathogenicity after this procedure.

Erythrogenic toxin production for the strains of streptococci described above was also studied by Kodama and his associates. Seventy-two and two-tenths per cent of Type 2, 91.6 per cent of Type 4, and 86.9 per cent of Type 8 strains formed this toxin. These differences are not as definite as those given above for mouse virulence. The ability of organisms of the various types to form this toxin has also been studied by Bailey (13). Only small numbers of strains were studied but this product was formed by streptococci of Type 3 much more frequently than by those of Types 4 and 6. These observations are probably statistically significant.

Relation of serological type to therapy. It has been amply shown by Loewenthal (21) and Lyons (22) that sera for the protection of experimental animals against hemolytic streptococcal infections must contain homologous type specific antibodies. This fact undoubtedly accounts for the failures of many early attempts at the preparation of therapeutic sera. Since the development of adequate methods of serological typing, type specific therapeutic sera have been prepared in animals but have received little clinical trial. Platou, Dwan and Hoyt (23) have described *in vitro* experiments which demonstrate a feeble antibacterial activity in type specific convalescent scarlet fever sera, and are investigating the use of such sera in the treatment of this disease.

No data are available, either of an experimental or clinical nature, which may be used to determine the response of infections due to the various types of hemolytic streptococci to the several sulfonamides.

METHODS

1. Isolation of organisms and Lancefield classification. All the strains of hemolytic streptococci described in this study have been isolated from individuals suffering from various types of hemolytic streptococcal infections or from the excised tonsils of normal persons. The procedures used in the isolation and Lancefield classification of these organisms have been described elsewhere (17). All strains were demonstrated to be members of Group A.

2. Griffith typing. Since the development by Griffith of an agglutination method for the division of strains of hemolytic streptococci of the Lancefield Group A into types, various modifications of his methods have been proposed. These have been directed at the preparation of better agglutinating suspensions and of sera more nearly free from intra-type cross reactions. Procedures used in this study will be described in detail:

a. Agglutinating suspensions. Two principal difficulties have been encountered in the preparation of satisfactory agglutinating suspensions: the first and most serious is the failure of the streptococci to grow diffusely in broth culture; the second is the tendency of certain strains to be inagglutinable even though the suspension is otherwise satisfactory. Repeated rapid transfer in liquid media or the addition of certain substances, such as serum, ascitic fluid (5), or live trypsin (24) to nutrient broth has been proposed for the elimination of granular suspensions. Considerable experience with each of these showed that none was entirely satisfactory. Serial passage in broth was exceedingly time-consuming; the addition of serum and of trypsin produced a very high percentage of inagglutinable suspensions.

Exceedingly satisfactory suspensions for the agglutination test were obtained if the cultures were incubated at a temperature below 30° C., as suggested by Ward and Rudd (6) but, under these conditions, growth of sufficient density developed very slowly. Usable suspensions have been secured within a reasonable period of incubation in the following manner: small tubes containing 2 to 3 cubic centimeters of tryptic digest broth (14) were inoculated with a large loopful of the organism to be studied, which had been freshly transferred on blood agar plates. The tube was closed with a sterile rubber stopper, and incubated in a rotating box at room temperature for 15 to 18 hours. Approximately 90 per cent of all strains yielded suspensions satisfactory for the slide agglutination test by this method.

A small number formed granular suspensions when cultivated in this way. These can be improved by adding a drop of trypsin solution (Difco) to the sediment and incubating for from 15 to 60 minutes at 37° C. The preparation must be examined frequently and typing carried out as soon as the granularity has disappeared, as prolonged incubation increases the incidence of inagglutinable suspensions.

Considerable numbers of strains which were untypable when first studied were readily typed after cultivation

and storage for several weeks or even months in nutrient agar slabs with agar seal at 5° C. Some of these strains were inagglutinable on the first examination but most of them tended to show agglutination in sera of several different types. This was especially true of a large number of strains of Type 12 which also agglutinated in Type 13 serum and strains of Type 11 which agglutinated in Type 12 serum. Nearly all of these were entirely type specific when retyped after storage in the manner described above. This phenomenon is probably related to the development in storage of glossy variants which Lancefield (25) has proposed as most suitable for typing by the agglutination technique because of their simpler antigenic structure.

b. Type sera. The typing sera used in this study were provided in crude undiluted form by the Lederle Laboratories and were prepared in rabbits. The titer of each was determined by a simple method. Undiluted serum was added, drop by drop, to 2 cubic centimeters of normal saline until agglutination of the homologous type was just observed, and then approximately 25 per cent more serum was added. Each dilute serum was then tested against suspensions of known Griffith type strains of hemolytic streptococci. Those showing cross reactions required absorption. This was best accomplished by the use of a non-type-specific variant of one of the ordinary type strains. Griffith (7) has described a method for the preparation of such a variant by the growth of Type 3 organisms upon agar containing homologous serum. Certain strains of Group A, however, which had been maintained on artificial culture media for a long period of time, showed colonies of more than one form when plated out. Suspensions prepared from a number of these usually yielded at least one which failed to agglutinate in the homologous serum. Such a strain was then cultured in large volumes of nutrient broth. After 18 hours of incubation the organisms were removed by centrifugation; those derived from 100 cubic centimeters were used for the absorption of 1 cubic centimeter of diluted serum. This operation was performed at 37° C. in a rotator. The serum was tested against agglutinating suspensions at hourly intervals and, when the cross reaction had been eliminated, the absorbing organisms were immediately removed in the centrifuge, the serum transferred to a sterile tube, and merthiolate added to a concentration of 1 to 10,000. Following such absorption a few sera still showed cross reactions for certain types. These were eliminated by absorption in the manner described above, using organisms of the type with which crossing occurred.

Much time was saved by preparing pools of sera of various types. It was advisable to combine in groups the sera for types among which cross agglutination occurred. Four sera selected in this way were mixed, diluted 1 to 2 and lightly absorbed with the organisms from 100 to 200 cubic centimeters of broth culture. Such pools, while not absolutely specific, usually limited the number of type sera required to 8 or less.

c. The slide agglutination test. Two cubic centimeters of a broth culture prepared as described above were

centrifuged and nearly all of the supernatant broth poured off. Drops of this thick suspension were placed with a platinum loop, 1.0 millimeter in diameter, upon a glass slide ruled into small squares by means of a wax pencil. The pooled sera were mixed with the drops of suspension by means of a 32 gage Nichrome wire loop, ½ to ¾ millimeter in diameter. The slide was rotated and then examined with a hand lens against a rather dark background and agglutination was observed in one or more pools. The specific sera contained in these pools were then set up in exactly the same manner with the suspensions.

This method has been most satisfactory and definite type specific agglutination was obtained with practically all of the typed strains. Repeated attempts were made to differentiate the several available strains of Types 4 and 24 by agglutinin absorption of the Lederle sera and sera obtained from Dr. Griffith. It was in no case possible to distinguish between these two types and, for the purpose of this study, strains agglutinating in Types 4 and 24 sera have been included in Type 4.

The procedures described have permitted the accurate classification of from 80 to 95 per cent of all strains. The number of typable strains appeared to vary with the source of the material.

SOURCE OF MATERIAL

A brief description of the type of clinical material from which the strains of hemolytic streptococci described in this study were isolated is pertinent.

Otitis Media. This group of 64 strains was isolated from materials obtained from the ears of children whose presenting complaint in the clinic, in nearly every instance, was referable to an infection of the middle ear or mastoid process. Many had a previous history of respiratory infection or sore throat, but none of scarlet fever.

Nasopharynx. The 28 strains included in this group were isolated from individuals who were suffering, or had shortly before suffered, a typical attack of tonsillitis.

Excised Tonsils. All tonsils excised in the clinic service of Stanford Hospitals since February, 1940, have been studied for the presence of hemolytic streptococci and the results described in detail elsewhere (17). All the Group A strains, which were isolated and typed by the methods described in this paper, have been included—112 in all. Strains isolated during the months of February to October, 1940, have been included under 1940, and those from October, 1940 to July, 1941, under 1941.

Scarlet Fever. All of the cases of scarlet fever admitted to the San Francisco Hospital from February, 1941 to July, 1941, were studied. Group A hemolytic streptococci were recovered in 15 of 17 cases.

Miscellaneous. This group of 41 strains includes 27 isolated from various surgical infections, usually cellulitis of an extremity. Also included are 3 obtained from the spinal fluid of meningitis cases, 7 from cases of pneumonia or acute bronchitis, 1 from puerperal sepsis and 3 cultured from the urine.

RESULTS

The results obtained in the serological typing by the Griffith method of strains of hemolytic streptococci isolated from various sources in 148 instances of hemolytic streptococcal infection and from the excised tonsils of 112 normal individuals are presented in Table II.

No strains of Types 3, 8, 14, 18, 19, 22, 26, 28, 29, or 30 were isolated. Types 7, 16, 20, and 21 are not members of Group A (14). The data presented under five headings based on the source of material, and the percentage values are calculated for each division separately. Totals are presented for 1940 and 1941 separately and combined with percentages calculated for each year and, in the last instance, for all strains.

Seasonal variations in types. Striking seasonal variations in the isolated types were noted. Types 1 and 2, which included 20.6 per cent of the isolated strains in 1940, were found in only 6.2 per cent of cases in 1941. Types 6 and 11 were more common in 1941, but two common types, 12 and 25, showed little variation in total number during the two seasons.

If the data for the 2 years are studied separately in relation to the source, which has been done but not included in the table, it is found

that Types 1 and 2, which were isolated from 31 per cent of cases of clinical infection in 1940, were present in only about 2 per cent in 1941. Practically all of the examples of these two types in 1941 were recovered from excised tonsils, where they had probably been resident since the previous season. Types 12 and 25 each occurred about twice, and Type 6 about 10 times as frequently in infectious states in 1941 as in 1940.

These variations are not nearly so striking if the strains from excised tonsils are considered for the 2 years. Types 1 and 2 appear only about one-third as frequently in 1941, but most of the other types were present in numbers of about the same order of magnitude in each year. Type 12 was present much less often in the tonsils in 1941, though more often a cause of infection than in the previous year.

Relationship of serological type to source of material. An examination of the composite data for the 2 years included in this study shows that the type distribution in strains from various sources is quite similar with a few notable exceptions. If the strains from otitis media, mastoiditis and tonsillitis be included together as examples of infection of the nasopharynx and its complications, and if the type distribution of the

TABLE II

Distribution of Griffith types among strains of Group A hemolytic streptococci isolated in the San Francisco area

Source	Number of strains		Griffith types																No type
			1	2	4	5	6	9	10	11	12	13	15	17	23	25	27		
Otitis media.....	64	Number Per cent	5 7.8	8 12.5	3 4.7	1 1.6	5 7.8		1 1.6	2 3.1	9 14.1		2 3.1		1 1.6	22 34.3	2 3.1	3 4.7	
Nasopharynx.....	29	Number Per cent	3 10.4	3 10.4	3 10.4		1 3.4		1 3.4		3 10.4				4 13.8	4 13.8	1 3.4	6 20.6	
Miscellaneous.....	41	Number Per cent	3 7.6	3 7.6	1 2.4	3 7.6	2 4.9	1 2.4			7 17.1	1 2.4	1 2.4	1 2.4	2 4.9	5 12.2	3 7.6	8 19.5	
Excised tonsils.....	112	Number Per cent	7 6.2	19 17.0	10 8.8	1 0.9	3 2.6		1 0.9	3 2.6	15 13.4	7 6.3	4 3.6		2 1.7	15 13.4	3 2.6	22 19.6	
Scarlet fever—1941....	15	Number Per cent		1 6.7	1 6.7					6 40.0					1 6.7	4 26.5		2 13.4	
Total—1940 (January to December)	147	Number Per cent	17 11.6	28 19.0	9 6.1	2 1.4	2 1.4		2 1.4	1 0.7	20 13.6	2 1.4	3 2.0	1 0.7	4 2.7	30 20.3	6 4.1	20 13.6	
Total—1941 (January to July).....	113	Number Per cent	1 0.9	6 5.3	8 7.1	3 2.6	9 7.9	1 0.9		10 8.7	9 7.9	7 6.2	4 3.5		6 5.3	21 18.6	3 2.6	25 22.1	
Total—1940 and 1941..	260	Number Per cent	18 6.9	34 13.1	17 6.5	5 1.9	11 4.2	1 0.4	2 0.8	11 4.2	29 11.2	9 3.5	7 2.7	1 0.4	10 3.8	51 19.5	9 3.5	45 17.3	

7 most frequent types be compared with that for the same types isolated from a group of infections, most of which are "surgical" in nature (Table III), it will be noted that, except for Type 25, the percentage incidence of the types in each group is very similar. The distribution of strains from excised tonsils is included in this table and shows a similar pattern in most instances.

TABLE III
Percentage of seven most common types in San Francisco by source

Source	Number of strains	Griffith types							Total
		1	2	4	6	12	23	25	
Otitis media and tonsillitis.....	74	7.8	10.6	5.8	5.8	11.6	4.9	25.0	71.5
Surgical infections....	41	7.6	7.6	2.4	4.9	17.1	4.9	12.2	56.7
Excised tonsils.....	112	6.2	17.0	8.8	2.6	13.4	1.7	13.4	63.1

The frequency with which Type 25 has been isolated from cases of otitis media during this survey is most striking. It is also important to note that it predominates in this manner only among the strains isolated from this disease.

Too small a number of examples of the various types of hemolytic streptococcal disease have been studied to draw conclusions in regard to the relationship of type to any of the other manifestations of infection due to this organism. It is worthwhile, however, to note that, in 3 cases associated with bacteremia, Types 1, 5, and 6 were involved, and that Type 5 was responsible for the 2 cases of meningitis studied. This type, while exceedingly uncommon, was therefore isolated from 3 of the 5 most seriously ill patients.

Relationship of source to typability. All of the strains of hemolytic streptococci described have been studied by identical methods and approximately the same incidence of untypable strains was found in the various groups, except those derived from ear and mastoid infections in which only 5 per cent were not classified as contrasted with about 20 per cent of all other strains. This very marked difference cannot be explained. It may be that more highly invasive strains lend themselves to typing more readily because of antigenic changes in the organism, or that these strains simply happen to be members of the described Griffith types more frequently than strains from other sources in the San Francisco area.

DISCUSSION

Technical difficulties, which may be largely avoided by procedures similar to those described in this paper, have prevented the widespread application of the Griffith method for the serological classification into types of hemolytic streptococci of the Lancefield Group A. This is unfortunate since these types have the same application in the study of hemolytic streptococcal infections as the pneumococcal typing offered in the investigation of infections due to these organisms.

From the data presented in this paper and from those of other workers, certain definite facts emerge. Recovery from Group A hemolytic streptococcal infections is associated with the development in the host of type specific antibodies. A small group of types is responsible for scarlet fever throughout the world, but there is adequate evidence that in any community the same types are regularly responsible for this disease, the incidence of individual types varying from year to year. It is not possible to determine at present why these variations should occur. It may be due either to shifting immunity in the potential host population, or to changes in the invasiveness of the organism in association with the production of infection. Coburn and Pauli (26) have studied certain aspects of the latter hypothesis and feel that recovery from infection of the nasopharynx in adults, but not in children, is associated with loss of infectiveness on the part of the hemolytic streptococcus. This work is entirely unconfirmed.

The same types responsible for scarlet fever may cause tonsillitis without rash. This important observation has finally settled the question as to the presence of a specific "scarlet fever streptococcus." It is obvious that the most important factor governing the production of the two diseases is the state of antitoxic immunity in the individual at the onset of the infection. The important differences in the nature of the organism must also exist as suggested by the numerous instances in which hemolytic streptococcal pharyngitis without rash has occurred one or more times in individuals who have later acquired scarlet fever.

Hemolytic streptococci of the types common responsible for scarlet fever may also cause surgical infections, but in most of the previous studies

they have not predominated as etiological agents in the latter conditions. It is of interest to point out that, among the strains described in this paper, the incidence of types in surgical and respiratory infections was similar. This was also true among strains collected in the Boston area (27).

In the United States, therefore, hemolytic streptococcal infections, regardless of the site of the disease process, are caused by similar small groups of types. Instances of sporadic infection due to types of streptococci not at that time commonly causing infection appear to be no more common among the group of "surgical" than among the respiratory infections.

The strains of streptococci isolated from the excised tonsils of healthy persons do not, season by season, mirror exactly the distribution of types responsible for infections during the same interval. This is to be expected since streptococci that have gained residence within the tonsils tend to remain there for long periods of time (17), so that strains collected from this source will be partly the residue of previous seasons and partly the result of invasion by types currently common in disease states. It seems almost certain that the tonsils of such carriers must be the reservoir from which infection spreads out into the community each season.

Many of the suppurative complications associated with and following hemolytic streptococcal pharyngitis and scarlet fever have been shown to be caused by reinfection with a type of streptococcus different from that present at the onset of the disease. This is particularly true of complications arising after the first week of the illness.

Some evidence exists that strains of different types vary in their virulence for mice and their toxigenicity. That certain types are more invasive in man is suggested by the fact that a few cause most of the clinical infections in the community, though many others may be demonstrated to be present in the throats of healthy carriers. It is also clear that some types are more apt to produce severe disease or to prepare the soil for complications involving cross infection by a streptococcus of another type. This is well demonstrated by the work of de Waal, and is suggested by the frequency with which Type 25 has been isolated from otitis media in this survey. Of

strains isolated from examples of this infection, this type was observed more than twice as frequently as were the strains of any other type. It is also important to note that Type 25 predominates in this manner only in this disease, suggesting that it was more likely to produce complications after invading the nasopharynx than the other types. It is possible that Type 25 was more commonly present in the community than the data obtained in this study indicate. This impression is supported in part by the fact that several of the small series of scarlet fever strains were members of Type 25. If this were so, then the other common scarlet fever type might have been expected to have appeared as a frequent cause of otitis media, which is not the case. This evidence, then, strongly suggests that strains of Type 25 were more actively invasive than those of other types and have continued to be so over a 2-year period.

Whether this enhanced pathogenicity is a permanent property of strains of certain types cannot yet be determined but it is extremely unlikely that any will prove to be consistently more virulent over wide geographic areas.

Such variations in the type and severity of disease, if confirmed and extended, might be of great value in determining prognosis and therapy of streptococcal infections. No definite evidence is available as yet bearing on the relationship of the serological type of hemolytic streptococcus to treatment with sulfonamides, but preliminary observations (28) suggest that there may not be any real differences in this respect among infections caused by the various types.

It has been possible to prepare satisfactory antitoxic antisera for the treatment of scarlet fever and other infections caused by the hemolytic streptococcus, since these substances are not related to serological type. Any sort of useful antibacterial antibody must, however, be type specific. For this reason it has been proposed (23) that convalescent scarlet fever sera be collected in type specific pools, the etiological organisms being typed before serum is administered. Such a method would be feasible because only a small number of types are isolated from cases of this disease during any season. Such sera would be of low antibody titer and their clinical value could be

determined only by trial. Suitable sera could probably be prepared in animals.

The importance of the application of serological typing to the study of the epidemiology of hemolytic streptococcal infections is obvious. The work of Colebrook (11) has shown the importance of carriers in the introduction of infection in the puerperal state, and Miles (18) has demonstrated that wound infections spread from patient to patient in the hospital ward and has indicated the rôle of attendants in this process. The importance of reinfection with new types in the development of complications in scarlet fever wards has been commented upon, and indicates the necessity for the isolation of such cases in wards according to the type of infecting streptococcus.

The study of epidemics of streptococcal infections by serological typing of the infecting organisms may lead to their more rapid control and to constructive plans for their prevention. This will be particularly important during war conditions when large groups of injured men will be concentrated in hospitals.

Much has been contributed to an understanding of hemolytic streptococcal infection by the application of serological typing but many questions remain unanswered. Most of these pertain to the relationship of the type of infecting organism to the nature, severity, prognosis, and response to therapy of the clinical disease. It is to be hoped that a widespread adoption of this method will soon lead to a further understanding of these common and often serious infections.

SUMMARY

1. Hemolytic streptococci of the Lancefield Group A may be readily classified by the agglutination method of Griffith.

2. Modifications of this method are presented which increase its reliability.

3. Data obtained by the application of these methods to strains of hemolytic streptococci isolated from 260 individuals are presented. One hundred and forty-eight of these were suffering from active infections; 112 were healthy carriers.

4. These data indicate that:

a. Hemolytic streptococci of a small number of types are responsible for nearly all of the infections in any season in San Francisco.

b. The same types do not predominate in every season.

c. The types predominating in respiratory infections are also those most commonly isolated from "surgical" infections and from the excised tonsils.

d. Type 25 was isolated more than twice as frequently from cases of otitis media than any other type and therefore appears to have been more invasive during this 2-year period than other types.

e. Strains from middle ear infections were more readily typable than those from other sources.

5. These observations are compared with those obtained by other workers and their significance is discussed.

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BIBLIOGRAPHY

1. Lancefield, R. C., A serological differentiation of human and other groups of hemolytic streptococci. *J. Exper. Med.*, 1933, 57, 571.
2. Hare, R., Sources of haemolytic streptococcal infection of wounds in war and civil life. *Lancet*, 1940, 1, 109.
3. Rantz, L. A., and Keefer, C. S., The distribution of hemolytic streptococci groups A, B and C in human infections. *J. Infect. Dis.*, 1941, 68, 128.
4. Sherman, J. M., The streptococci. *Bact. Reviews*, 1937, 1, 1.
5. Griffith, F., The serological classification of streptococcus pyogenes. *J. Hyg.*, 1935, 34, 542.
6. Ward, H. K., and Rudd, G. V., Studies on haemolytic streptococci from human sources; cultural characteristics of potentially virulent strains. *Australian J. Exper. Biol. and M. Sc.*, 1938, 16, 181.
7. Neisser, H., Serological typing of streptococcus pyogenes and its application to certain infective conditions. *J. Path. and Bact.*, 1939, 48, 55.
8. Griffith, F. W., and Allison, V. D., Annual Report of the Chief Medical Officer to the Ministry of Health, 1936.
9. Green, C. A., Serological types of hemolytic streptococci in an epidemic of scarlatina. *J. Hyg.*, 1937, 37, 318.
10. de Waal, H. L., The serological types of haemolytic streptococci in relation to the epidemiology of scarlet fever and its complications. *J. Hyg.*, 1940, 40, 172.
11. Colebrook, Dora C., The source of infection in puerperal fever due to hemolytic streptococci. *Med. Res. Council. Special Report Series*, No. 205, London, His Majesty's Stationery Office, 1935.

12. Shaw, C., Serological grouping and typing of haemolytic streptococci from various human sources. *Lancet*, 1937, 2, 1193.
13. Bailey, J. H., The types of hemolytic streptococci found in scarlet fever patients and in the throats of grammar school children. *Am. J. Hyg.*, 1939, 29, 107.
14. Pauli, R. G., and Coburn, A. F., Studies on the serological typing of streptococcus hemolyticus. *J. Exper. Med.*, 1937, 65, 595.
15. Keogh, E. V., Simmons, R. T., and Wilson, H., *Streptococcus pyogenes* (Group A). A review of 1,341 strains collected from various human sources. *Australian J. Exper. Biol. and M. Sc.*, 1941, 19, 51.
- 16a. Kodama, T., Tiku, Y., Kodaira, T., and Kodama, A., The serological grouping and typing of the hemolytic streptococci isolated in Tokyo. I. *Kitasato Arch. Exper. Med.*, 1937, 14, 245.
- b. Kodama, T., Ozaki, M., Nisyama, S., and Tiku, Y., The serological grouping and typing of the hemolytic streptococci isolated in Tokyo. II. *Ibid*, 1938, 15, 162.
- c. Kodama, T., Ozaki, M., Nisiyama, S., Igarasi, J., Tiku, Y., and Kawamura, H., The serological grouping and typing of the hemolytic streptococci isolated in Tokyo. III. *Ibid*, 1939, 16, 110.
17. Rantz, L. A., The hemolytic streptococci. Studies in the carrier state in the San Francisco area. *J. Infect. Dis.*, 1941, 69, 248.
18. Miles, A. A., Schwabacher, H., Cunliffe, A. C., Ross, J. P., Spooner, E. T. C., Pilcher, R. S., and Wright, J., Hospital infection of war wounds. *Brit. M. J.*, 1940, 2, 855.
19. Kuttner, A. G., and Krumwiede, E., Observations on the effect of streptococcal upper respiratory infections on rheumatic children: A three year study. *J. Clin. Invest.*, 1941, 20, 273.
20. Brown, W. A., and Allison, V. D., Carriers and return cases in scarlet fever. *J. Hyg.*, 1935, 35, 283.
21. Loewenthal, H., Type-specific and group-specific sera against streptococci. *Brit. J. Exper. Path.*, 1934, 15, 298.
22. Lyons, C., Immunotransfusion and antitoxin therapy in hemolytic streptococcus infections. *J. A. M. A.*, 1935, 105, 1972.
23. Platou, E. S., Dwan, P. F., and Hoyt, R. E., Streptococcus convalescent serums (scarlatinal). The potentialities of type specific pools. *J. A. M. A.*, 1941, 116, 11.
24. Coburn, A. F., and O'Connell, S., Advances in serological typing and of streptococcus haemolyticus. *Proc. Soc. Exper. Biol. and Med.*, 1939, 40, 645.
- 25a. Lancefield, R. C., Type specific antigens of Matt and Glossy variants of Group A hemolytic streptococci. *J. Exper. Med.*, 1940, 71, 521.
- b. The significance of M and T antigens in the cross reactions between certain types of Group A hemolytic streptococci. *Ibid*, 1940, 71, 539.
26. Coburn, A. F., and Pauli, R. H., Interaction of host and bacterium in the development of communicability by streptococcus haemolyticus. *J. Exper. Med.*, 1941, 75, 551.
27. Keefer, C. S., Rantz, L. A., Shuman, H. H., and Rammelkamp, C. H., The distribution of hemolytic streptococci in 819 cases of infection. *Arch. Int. Med.* (In press.)
28. Rantz, L. A., Unpublished observations.

CARDIAC OUTPUT, BLOOD AND INTERSTITIAL FLUID VOLUMES, TOTAL CIRCULATING SERUM PROTEIN, AND KIDNEY FUNCTION DURING CARDIAC FAILURE AND AFTER IMPROVEMENT¹

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The relation of cardiac output, blood and interstitial fluid volumes, plasma protein concentration, and kidney function in heart failure is still a subject of investigation. The majority of investigators in this country (1, 2) and in England (3, 4) have believed that the fundamental factor in heart failure is a diminished cardiac output for a given diastolic size against a given resistance, and that increase in venous pressure and blood volume are secondary phenomena, although themselves responsible for many of the symptoms of heart failure. Diminished cardiac output would first be manifested by failure to meet the oxygen requirements of the body during exercise, and only in extreme cases by a subnormal cardiac output at rest. Thus, measurements of cardiac output at rest would only be below normal in patients who showed symptoms of heart failure at rest. Since, however, even in this group Harrison (5) found the average values for cardiac output, as well as the range, similar to those in the compensated group, he concluded that diminished cardiac output was not an important factor in cardiac failure and developed his "Backward Failure" theory, attributing the major phenomenon of cardiac failure to increased venous pressure proximal to the failing (but still normally working) chamber.

The oliguria and nitrogen retention of cardiac failure are attributed to passive congestion by Harrison (6) and to "extrarenal" deviation of water by Fishberg (7). Neither of these explanations is satisfactory. Increase in renal venous pressure only results in oliguria when renal blood flow is reduced. If blood flow is unchanged, increase in renal venous pressure results in diuresis (8). If the oliguria is due to diminished water available

for excretion, this must be caused either by reduction in renal blood flow or increase in plasma protein concentration. The latter is contrary to observation.

Because of the complexities of cardiac failure, and adventitious phenomena such as elevation in metabolic rate, psychic disturbances, etc., it would seem that data obtained on the same patient when in failure and when compensated are more readily interpreted than statistical conclusions on different groups. Stewart and Cohn (1) studied the effect of digitalis in this way and found an increase in cardiac output and decrease in heart size with compensation. Friedman, Clark, Resnik, and Harrison (5) found no consistent change in cardiac output, stroke volume, arteriovenous oxygen difference, or basal metabolic rate with clinical improvement. Grollman's acetylene method (9) was employed in both investigations to measure cardiac output.

The present report concerns measurements of cardiac output, blood volume, interstitial fluid volume, plasma proteins, renal function, and other aspects of the circulation in a small group of patients when suffering from cardiac failure and again after restoration of compensation.

The patients all had definite congestive heart failure due to hypertensive or arteriosclerotic heart disease. Patients with aortic insufficiency or auricular fibrillation were not considered suitable for this study because of the method employed for estimating cardiac output. The first series of observations was made as soon as possible after admission before treatment, save for indicated sedatives and hypnotics. Estimations of cardiac output under standard conditions of bed rest and no food for fourteen hours were made on one morning, followed by estimations of blood and interstitial

¹ The expenses of this investigation were defrayed by a grant from the Commonwealth Fund.

fluid volumes, etc. The following day measurements of kidney function were carried out. The patient was then given full doses of digitalis and, if necessary, additional diuretics, and the observations were repeated when the subject was free from edema and other symptoms and was at rest or up and about the ward.

The data are presented under three headings: I. Cardiac output, blood pressure, and venous pressure. II. Blood and interstitial fluid volume, plasma protein concentration, and total circulating plasma proteins. III. Kidney function.

I. Cardiac output

In order to avoid the necessity of the patient's cooperation and the uncertainty of obtaining equilibrium in a bag-lung system before re-circulation, cardiac output was estimated by Bazett's method (10). While the constants utilized are empirically derived, the cardiac index of normal individuals by this method checks closely with the acetylene method which gives a value of 2.2 ± 0.3 for normal subjects under basal conditions. Since the same values for aortic area and artery length were used for calculation of output before and after compensation, an error in these might affect the absolute, but not the relative values.

Bazett's method is based on von Recklinghausen's principle that "the volume of blood leaving the arterial tree, and the distensibility of the system are the sole determinants of the fall in pressure in diastole." Since the blood leaving the arterial tree in the "systolic" period should be related to that leaving in diastole, according to the relative pressures in and the relative duration of these periods, calculation of the volume leaving in the "systolic" period is possible (10) if we accept the data of Whittaker and Winton (11). The total volume or stroke volume is then easily obtained.

1. Vd (blood leaving in diastole)

$$= \frac{12.7}{100} \times Z-D \times \left[\frac{V^1}{v_1^2} + \frac{V^2}{v_2^2} + \frac{V^3}{v_3^2} + \frac{V^4}{v_4^2} \right]$$

2. Vs (blood leaving in systole) = $Vd \times \frac{s}{d} \times \frac{Ms - 20}{Md - 20}$

3. Vt (stroke volume) = $Vs + Vd$

4. C.O. (cardiac output) = $Vt \times$ pulse rate

5. C.I. (cardiac index) = $\frac{\text{C.O.}}{\text{S.A.}}$

In these formulae, the following representations exist: Z = dicrotic pressure; D = diastolic pressure; $V^1, V^2,$

etc. = vessel volumes assumed by calculations from surface area and aortic areas; $v_1, v_2,$ etc. = pulse velocities for the corresponding volume sections; s and d are "systolic" and "diastolic" periods; Ms and Md represent the mean pressures of the respective s and d periods.²

To obtain $S, Z,$ and D pressures, a one- or three-bag compression system recording optically was first used. At these pressures, various criteria became apparent on the recorded volume brachial pulse curve. The value assigned to Z was calculated from the ratio of the heights of perpendiculars from the point of maximum elevation and from the top of the dicrotic notch. Later, in order to record more accurately all pressures, the bag-compression system was replaced by direct brachial arterial puncture under local anesthesia with a Gregg (12) needle optical manometer. A short number 22 needle was employed. That this arterial puncture did not disturb the patients is attested to by the fact that pulse rates taken during the procedure showed no, or only very slight, elevation over those recorded during the taking of pulse wave velocities.

Vessel volumes $V^1, V^2,$ etc., were computed from formulae utilizing Bazett's graph of aortic area, surface area, and height. Weights used were taken in the edema-free state and used in all calculations of output on the same patient.

For recording pulse wave velocities $v_1, v_2,$ etc., simple cup or glycerine tambours were held or fastened over the appropriate vessels and cardiac area. Each tambour was connected by equal length rubber tubing to segment capsules held in an Everbach triple segment capsule holder, avoiding parallax. Projection distance from capsules to recording camera was 1.25 to 1.5 meters. The actual distances traveled between tambours on the vessels were calculated by use of other predetermined constants (10).

Since the accuracy of the method depends considerably on the identification in the records of the start of ventricular discharge, heart sounds were substituted for sternal pulsations, but recordings of the electrocardiogram, apex beat, and heart sounds simultaneously with the subclavian pulse were made in many instances. The difficulty of denoting time of ejection is great, but the same criteria in the same patient were used at all times, although these varied in configuration among the individual patients.

The "diastolic" period was measured from the top of the dicrotic wave to the end of the pulse cycle; the rest was considered systolic.

Mean pressures were calculated from the brachial pressure curves by weighing or by use of a planimeter. All pressures recorded represent the mean of from 6 to 54 determinations, the greater number being those of the pressure pulses derived from direct arterial puncture. Pulse wave velocities are expressed as means of from 5 to 10 determinations.

Venous pressures were determined by using the method and auricular reference point of Lyons, Kennedy, and Burwell (13).

² It is advised that for full consideration in the use of this method Bazett's original paper be consulted.

TABLE I

Comparison of cardiac output estimated by Bazett's method and by acetylene method, and agreement of Bazett's method on different days

Patient Date	Age S.A.	P	S	Z	D	Z-D	M.P.	PV ₁	PV ₂	PV ₃	S.V.	C.O.	C.I.	R	Acetylene	
															C.O.	C.I.
1.	25															
September 11, 1939	1.92	51	102	76	51	25	75	3.5	5.9	4.4	85	4.3	2.3	98	4.6	2.4
2.	27															
October 21, 1939	2.25	60	115	97	61	36	78	4.8	5.2	7.6	86	5.2	2.3	102	5.7	2.5
3.	28															
September 22, 1939	1.74	60	111	97	69	28	92	5.0	4.7	7.3	67	4.0	2.3	120	4.0	2.3
		62	94	76	51	24	76	4.2	4.0	5.5	59	3.6	2.1	109	4.0	2.3
4.	45															
February 12, 1941	1.55	76	116	77	58	19	79	5.0	5.7	7.0	41	3.1	2.1	113		
5.	17															
April 10, 1941	1.76	81	136	92	77	15	99	5.3	5.2	6.5	38	3.1	1.8	165		
April 23, 1941		81	139	100	81	19	101	5.0	5.7	5.8	40	3.3	1.9	160		
May 3, 1941		81	134	94	77	17	97	4.5	5.3	6.3	40	3.3	1.9	153		
6.	28															
November 1, 1939	1.67	67	110	93	71	22	95	4.0	6.8	7.2	49	3.3	2.0	140		
November 5, 1939		65	104	95	66	29	90	3.7	6.8	7.8	48	3.1	1.9	142		
January 12, 1940		59	104	90	64	26	86	3.5	5.3	6.5	57	3.4	2.0	129		
May 1, 1940		61	106	93	68	25	88	3.9	6.3	7.0	43	2.7	1.6	165		

The following abbreviations are used in this and subsequent tables: S.A. = surface area in *square meters*; P = pulse rate *per minute*; S = systolic blood pressure in *mm Hg*; Z = diastolic blood pressure; D = diastolic blood pressure; M.P. = mean blood pressure; PV₁ = pulse wave velocity heart to subclavian artery; PV₂ = pulse wave velocity heart to femoral artery—PV₁; PV₃ = pulse wave velocity subclavian to brachial arteries; S.V. = stroke volume in *cc. per beat*; C.O. = cardiac output in *liters per minute*; C.I. = cardiac index, *liters per square meter*; R = effective peripheral resistance in arbitrary units.

Although this method of measuring cardiac output is physiological in its principles, it is entirely empirical in its application as all constants used have been adjusted by Bazett to give good agreement in normal subjects with values of cardiac output determined simultaneously with acetylene. The results in our hands on normal adults are shown in Table I. The table also shows the agreement in estimated cardiac output in two subjects on different days and in the last subject at longer intervals.

Three patients with thyrotoxicosis were also studied before and after thyroidectomy to test the method's ability to detect changes known to occur

in this disease (9). These data are presented in Table II. Values of cardiac output were all above normal during the active phase of the syndrome, the elevation being dependent upon the tachycardia as the stroke volume remained the same.

The data from the six cardiac patients are given in Table III. All of the six patients showed significant reductions in stroke volume and minute output while in failure. The pulse rates were all faster during failure, but in only one was the rate above 90.

From the data in Table III, the mechanism of the reduced output in failure is evident. While the pressure difference in the *d* period (Z-D) was

TABLE II
Estimations of cardiac output before and after thyroidectomy for hyperthyroidism

Patient Age		P	S	Z	D	Z-D	PV ₁	PV ₂	PV ₃	S.V.	C.O.	C.I.	R	BMR
1.	Before	107	103	93	73	20	4.6	5.2	6.6	49	5.3	3.3	89	+54
22	After	73	135	98	73	25	4.6	4.9	5.3	50	3.7	2.3	126	+6
2.	Before	97	115	79	60	19	4.5	6.9	7.3	46	4.5	3.0	81	+42
49	After	68	129	98	72	26	4.7	6.2	9.1	46	3.1	2.1	136	+15
3.	Before	124	114	76	61	15	4.6	5.1	6.8	40	5.0	2.9	86	+60
30	After	77	123	85	67	18	4.5	6.2	7.5	38	2.9	1.7	159	+5

BMR = basal metabolic rate.

somewhat reduced in all but Patient 1, the major change occurred in the pulse wave velocities, which were uniformly increased during failure.⁸ Velocities after recovery became slower in both the larger and smaller vessels.

The blood pressure changes are of interest. In all but Patient 3, the diastolic pressures were lower after recovery. Systolic pressures likewise fell in four, but rose in two. Since the mean pressure is determined by the cardiac output and the mean

⁸ Except for PV₂ in Patient 2, the difference in velocity during and after failure is at least seven times the standard error of the difference.

peripheral resistance, this latter may be calculated from the relation of mean pressure to cardiac index

$$\left(R = K \frac{M}{C.I.} \right).$$

If K be assigned an arbitrary value of 3, the values for normal young adults range from 80 to 120 (10). The calculated effective resistances were much increased in failure compared to their recovery levels. The decrease in cardiac output and the increase in mean pressure both contribute to the increase in the ratio.

TABLE III
Changes in pulse rate, blood pressure, and cardiac output during congestive heart failure and after recovery of compensation

Patient Age		P	S	Z	D	MP	Z-D	PV ₁	PV ₂	PV ₃	S.V.	C.O.	C.I.	R	V.P.
1. F. T.	Before	80	240	205	144	168	61	9.6	17.3	16.9	28	2.3	1.3	455	35
66	After	59	172	143	83	128	62	4.8	12.0	12.0	66	3.9	2.3	170	10
2. P. K.	Before	74	151	113	81	109	32	5.2	10.9	10.8	39	2.9	1.6	206	35
70	After	54	141	110	70	100	40	4.3	10.9	8.6	71	3.9	2.1	144	7
3. J. F.	Before	84	151	125	87	121	38	8.4	10.1	12.1	30	2.5	1.6	232	20
72	After	70	179	138	85	125	53	6.1	8.1	10.4	50	3.5	2.2	173	7
4. W. T.	Before	81	163	142	111	140	31	4.8	9.8	15.8	34	2.7	1.8	240	25
50	After	64	135	118	83	108	35	3.8	6.6	9.0	57	3.6	2.4	137	6
5. M. A.	Before	97	147	126	102	123	24	5.9	9.7	8.7	33	3.1	1.7	190	26
70	After	82	125	104	77	101	27	5.2	7.0	7.2	47	3.9	2.2	138	12
6. J. Y.	Before	81	136	108	78	101	30	8.8	7.6	10.0	26	2.1	1.3	234	30
65	After	63	145	94	56	84	38	6.3	5.9	7.0	61	3.9	2.4	105	10

V.P. = venous pressure in *cm. saline*.

Venous pressures were markedly elevated during failure in all patients, and in those in whom circulation times were done these were likewise prolonged. The size of the heart was larger during failure in the four patients who had x-ray studies before and after failure.

In this group of patients, cardiac insufficiency was manifested by marked reduction in stroke volumes and significant decreases in the total minute discharge in spite of higher pulse rates. These changes have been calculated from the data of peripheral phenomena accompanying this state. The total "run-off" of blood from the arterial tree was less in failure in spite of higher blood pressures. This is reflected in the reduced elasticity of the larger central and peripheral arteries which are more distended at the higher pressures and in the lower effective pressure head in the *d* period (*Z-D*) (14).

That peripheral vasoconstriction was present must be inferred since both diastolic pressures and the effective peripheral resistances (*R*) were higher during failure. This vasoconstriction would actually reduce the total arterial cross-sectional area in spite of distention of the large arterial trunks.

Although the volume of blood discharged per beat was lower during failure and was uncompensated by a rise in pulse rate, the total volume of blood in the vascular system was greater (see Section II). With reduced capacity of the total arterial tree, this excess volume must have been accommodated within the venous system.

It seems improbable that sufficient blood could be transferred to the distensible venous system (15) by arteriolar constriction to account for the elevated venous pressures during failure if blood volume were normal. The increased blood volume, rather than redistribution of a constant volume of blood, would seem to be the major factor in producing the elevated venous pressure (16). This hypothesis is supported by another patient with coronary occlusion and minimal congestive failure who showed no change in venous pressure or in blood volume with improvement. At the first determination her cardiac index was 1.65, peripheral resistance 167, and venous pressure 12 cm. of saline. After improvement, her cardiac index was 2.2, peripheral resistance 130, and venous pressure 14 cm. saline.

These findings are consistent with Harrison's assignment of the symptoms of congestive failure to increased ventricular diastolic resistance, leading to retrograde stasis. Such increased ventricular resistance can only be due to pericardial adhesions, incomplete diastolic relaxation, or to blood remaining in the ventricle from incomplete emptying. There is no evidence for the first two of these. Our findings differ from Harrison's in the consistency of a diminished cardiac output during failure.

II. Variations in blood and extracellular fluid volumes and in plasma proteins

It has been shown by several investigators (17 to 25) that the majority of patients in cardiac failure have a reduced quantity of protein per unit volume of serum. Of 261 cases culled from the literature and other sources, 212, or 81.2 per cent, showed a total serum protein of less than 6.6 grams per 100 cubic centimeters, with a mean total serum protein of 5.67 grams per 100 cc. The reduction is due almost exclusively to a drop in serum albumin; of 127 cases in which the protein fractions were determined (17, 19, 20, 21, 22, 25), 117, or 92.6 per cent, showed a serum albumin of less than 4.6 grams with a mean serum albumin concentration of 3.41 grams per 100 cc.

Three primary factors could be responsible for a reduction of serum protein: inadequate protein intake, excessive protein loss, and failure of protein synthesis (26). All three of these may operate in cardiac failure. Furthermore, it has never been shown whether the reduction of serum albumin in cardiac failure is real or only apparent since an increase in serum volume regularly occurs in this condition (27) with possible dilution of serum protein. Patients who are suffering from their first bout of cardiac failure and who have been eating ordinary diets do not usually experience a prolonged anorexia preceding failure, so that inadequate protein intake would seem improbable as a major cause of hypoproteinemia; the same thought holds for impairment of protein synthesis. The loss of protein in the urine of these patients is usually slight. Two other possibilities remain: loss of protein into tissue spaces, and blood dilution. Attention was therefore directed toward these two factors.

The following determinations were made on each patient during the phase of cardiac failure and at maximal recovery: total blood volume, serum volume, interstitial fluid volume, hematocrit, serum proteins, and edema fluid proteins.

Total blood volume and serum volume were determined by the Evans Blue dye method as modified by Gibson and Evans (28). A Pulfrich photometer with an S-62 filter and a cell 1 cm. thick was used for estimations of the concentration of dye in the serum.

Extracellular fluid volume was determined by the thiocyanate method of Laviates *et al.* (29). Potassium thiocyanate was given by mouth twenty-four hours before the determination. The concentration of thiocyanate in serum was determined with a Pulfrich photometer, using an S-47 filter and a cell 1 cm. thick. In most instances, the concentration of thiocyanate in extracellular fluid itself was also determined.

Hematocrits were determined in the Wintrobe tube, using either heparin or a mixture of ammonium and potassium oxalate, 2 mgm. per 1 cc. of blood, as anticoagulant, and centrifuged until the volume of cells was constant. Serum proteins were determined by Robinson's modification (30) of Howe's micromethod. Extracellular fluid proteins were determined in the same way. All

specimens were done in triplicate. Extracellular fluid was obtained by means of Southey tubes. All blood was taken under oil and without stasis.

The data are given in Table IV. In all patients, total blood and serum volume values were increased during cardiac failure, the mean change in total blood volume with compensation being 1.15 liters, or 25.0 per cent. Gibson and Evans (27), studying a similar group of patients, found a mean increase of 22.3 per cent. They found that in extreme decompensation the mean increase was 55.3 per cent; but among these the distribution was very wide, eight out of fourteen showing an increase in blood volume of less than 35 per cent. In Gibson and Evans' series, red cells and serum participated equally in the increase, but this was not so in our cases since four out of six showed a considerable rise in hematocrit with compensation, and the increase of serum volume in failure was relatively much greater than total blood volume, averaging 0.92 liter, or 35 per cent. Blood volumes in the compensated phase were normal

TABLE IV
Summary of data on cases

Case	Weight	Blood volume	Serum volume	Total extracellular fluid	Serum total protein	Serum albumin	Serum globulin	Edema fluid protein	Hematocrit
	<i>kgm.</i>	<i>liters</i>	<i>liters</i>	<i>liters</i>	<i>grams per cent</i>				
1. F. T. Onset.....	81.4	6.60	3.82	31.8	6.98	4.26	2.72	0.18	42
Recovery.....	70.0	5.30	3.20	16.9	7.00	3.69	3.31		40
Difference.....	11.4	-1.30	-0.62	-14.9	+ .02	-0.57	+0.59		
2. P. K. Onset.....	87.0	5.88	3.88	32.9	6.95	4.57	2.38	0.67	34
Recovery.....	75.5	5.43	3.04	21.7	7.18	4.41	2.77		44
Difference.....	11.5	-0.45	-0.84	-11.2	+0.23	-0.16	+0.39		
3. J. F. Onset.....	61.4	5.23	3.97	26.9	6.07	3.39	2.68	0.96*	24
Recovery.....	50.8	3.96	2.57	18.6	7.50	4.21	3.29		35
Difference.....	10.6	-1.27	-1.40	- 8.3	+1.43	+0.82	+0.61		
4. W. T. Onset.....	72.0	5.75	3.50	38.8	6.57	3.81	2.76	0.39	39
Recovery.....	51.7	4.20	2.35	18.8	7.35	3.84	3.51		44
Difference.....	-20.3	-1.55	-1.15	-20.0	+0.78	+0.03	+0.75		
5. M. A. Onset.....	100.5	5.75	2.88	38.6	6.42	4.34	2.08	0.85	49
Recovery.....	73.5	5.00	2.35	20.8	7.52	4.70	2.82		53
Difference.....	-27.0	-0.75	-0.53	-17.8	+1.10	+0.36	+0.74		
6. J. Y. Onset.....	70.4	5.33	3.26	28.5	6.04	3.97	2.07	0.25	39
Recovery.....	54.4	3.67	2.29	19.1	6.73	4.16	2.57		38
Difference.....	-16.0	-1.66	-0.97	- 9.4	+0.69	+0.19	+0.50		

* Pleural fluid.

when compared with the values of Gibson and Evans (28).

Extracellular fluid volume decreased with recovery in all cases, the mean change being 12.73 liters. The mean value of total extracellular fluid in the recovery phase was 32 per cent of total body weight—still almost 50 per cent above normal. Decrease in weight occurred with recovery in all of the patients, the mean loss being 15.6 kgm.

Serum protein values, in terms of grams per 100 cc. of serum, increased with recovery in all cases, the increase being ascribable mainly to the globulin fraction. This change is only in concentration, for when total circulating serum proteins are calculated, the amount of serum albumin actually decreased markedly and the serum globulin decreased slightly.

The changes observed in blood volume are similar to those found in previous studies on similar cases (27). The increase in blood volume in our cases was more of a "hydraulic plethora" than in the cases of Gibson and Evans, as in our cases serum volume was the maximum participant in this change. Presumably, the vascular bed, mainly the venous side, increases in capacity to accommodate this increased volume (15).

Similarly, the changes in total extracellular fluid and weight are those commonly observed in recovery from cardiac failure. Although the two were generally in agreement, the apparent discrepancies are difficult to explain.

As noted, the final value of total extracellular fluid averaged 32 per cent of body weight, even though the patients showed no demonstrable edema. It is unlikely that this can be accounted for wholly on the basis of cardiac insufficiency, since by all other criteria cardiac compensation was much improved. We feel that this retention of fluid may be a peripheral effect for two reasons: (1) although anoxia causes an increase in capillary permeability (31), the time required for recovery may be prolonged; (2) the protein left in extracellular space may be picked up much more slowly via the lymphatics than water is by the capillaries. In the latter event, effective osmotic "pull" of water back into the blood stream would be reduced, since the remaining protein in extracellular space would exert a counter pressure.

Although serum proteins rose in value in terms of grams per 100 cc., actually the amount of

circulating protein decreased. In Table V, the total amounts of albumin and globulin, calculated by multiplying grams per cent by serum volume, are presented at onset and with recovery and the difference in these two values is recorded.

TABLE V
Total circulating proteins in failure and compensation with net changes

Case	Decompensation		Compensation		Net change	
	Total albumin	Total globulin	Total albumin	Total globulin	Albumin	Globulin
	grams	grams	grams	grams	grams	grams
1.	162.5	105.0	118.0	106.0	-44.5	+ 1.0
2.	176.0	92.5	134.0	84.3	-42.0	- 8.2
3.	134.5	106.0	108.0	84.5	-26.5	-21.5
4.	133.0	97.0	90.0	82.5	-43.0	-15.5
5.	125.0	60.0	111.0	66.5	-14.0	+ 6.5
6.	129.5	67.5	95.5	59.0	-34.0	- 8.5
Mean	143.4	88.0	109.4	80.46	-34.0	- 7.7

It is thus evident that, although total circulating albumin falls, the loss of water from the blood stream more than compensates, so that a normal concentration is approached. Now, when the amount of albumin available from extracellular fluid is calculated by multiplying its concentration per liter by the loss of extracellular fluid in liters, an average value of 73.9 grams is obtained. Since the average loss in the blood stream itself is 34.0 grams, this, added to the amount available from decreased extracellular fluid, gives a mean total of 99.9 grams unaccounted for, a value of 3.63 grams per each 100 cc. of blood. Such a quantity of protein cannot remain in extracellular fluid in the recovery phase. Urinary loss of proteins in these patients was negligible. Since nitrogen balances were not done, we have no idea how much was metabolized but the changes noted are in harmony with the work of Madden and Whipple (32), which shows that serum proteins are in a state of flux. Madden and Whipple have stated that "The tacit assumption has been that, once formed, unless obviously lost by . . . transudation . . . plasma protein is static. . . . A steady state of ebb and flow exists between it and a portion of the cell and tissue body protein." It is inconceivable that as much as 150 grams of protein (Case 5) could be left in extracellular space for any length of time, especially with reduction in volume of extracellular fluid. Ap-

parently, this large amount of protein, which was returned to the blood stream via the lymphatics, is stored for future use, presumably in the liver and reticulo-endothelium. Such a concept is wholly in accord with Whipple's work on plasmapheresis. This "dynamic equilibrium" of serum proteins holds not only for slow changes but for rapid changes as well. Stewart and Rourke (33), studying changes in sixteen patients undergoing ether anaesthesia and surgical procedures, showed a mean reduction of plasma volume of 13.8 per cent and a mean increase in extracellular fluid of

drawn without stasis at the start of the first period of urine collection and at twenty-minute intervals thereafter until the close of the experiment. Mid-point concentrations were determined by interpolation, and mean concentration for each period was measured by the use of a planimeter.

Inulin concentration in plasma and urine was measured by the method of Corcoran and Page (34). Phenol red concentration was determined in alkalized samples of plasma and urine by the use of a Pulfrich photometer, with an S-53 filter. Occasional minimal hemolysis was corrected by using an S-43 filter which shows no light absorption with phenol red in the concentrations used but which shows 2.5 times as much absorption with alka-

TABLE VI
Measurements of renal function in congestive failure and after compensation

Case	Blood urea nitrogen	Maximum specific gravity	Inulin clearance	Phenol red clearance	Urea clearance	Phenol red Inulin	
					<i>per cent of normal</i>		
1. F. T.	23.2 18.4	1.030 1.028	107 91	144 238	36 67	1.3 2.6	In failure. Compensated.
2. P. K.	18.6 18.1	1.023 1.020	61 83	75 280	56 78	1.2 4.4	In failure. Compensated.
3. J. F.	13.0 25.9	1.019 1.024	69 88	150 191	69 74	2.2 2.2	In failure. Compensated.
4. W. T.	18.5 25.3	1.017 1.020	74 68	155 228	67 39	2.1 3.3	In failure. Compensated.
5. M. A.	10.5 23.7	1.022 1.022	95 105	178 311	64 37	1.9 3.0	In failure. Compensated.
6. J. Y.	18.0 13.5	1.021 1.016	40 87	85 238	36 59	2.1 2.7	In failure. Compensated.

27.5 per cent; the mean reduction in plasma protein concentration, however, was only 0.5 gram per cent.

III. Kidney function

Kidney function during decompensation and after recovery was estimated by inulin, phenol red and urea clearances, and maximum specific gravity.

In the clearance studies, three or more urine specimens were collected at thirty-minute intervals by means of an indwelling urethral catheter. At the end of each period of urine collection, the bladder was washed with a measured amount of normal saline. One-half hour before the start of the first period of urine collection, 150 cc. of a 4 per cent solution of inulin were given intravenously. At the same time, 3 cc. of a 10 per cent solution of phenol red were given intramuscularly. In this manner, the clearances were done upon a falling blood concentration of the injected substances. Venous blood was with-

line hematin as does the S-53 filter. Urea content of urine was determined by the manometric urease method of Van Slyke (35) and that of blood by the hypobromite method of Van Slyke and Kugel (36). Plasma concentrations of inulin ranged from 61 to 10 mgm. per 100 cc., and of phenol red from 1.17 to 0.24 mgm. per 100 cc. These concentrations of phenol red are below those at which "self depression" of the clearance takes place (37).

The changes in maximum specific gravity, mean inulin, phenol red, and urea clearances in these patients are shown in Table VI. For purposes of discussion, the inulin clearance may be regarded as a measure of the volume of glomerular filtrate and the phenol red clearance as a measure of renal blood flow, or "effective" renal blood flow in Smith's terminology (37). Smith has abandoned the phenol red clearance in favor of the diodrast clearance for this purpose since the latter has the

higher plasma clearance (38). Both substances are excreted chiefly by secretory activity of the tubule cells, and both show depression of clearance values with increase in blood concentration. Since, however, Smith finds a fairly constant ratio between diodrast and phenol red clearances in the normal kidney when the plasma levels are low, it seems legitimate in this instance to use the latter as an indication of changes in renal blood flow. The presence of appreciable numbers of intrarenal arteriovenous anastomoses described by Spanner (39) would, of course, invalidate this assumption if these were uniformly open during decompensation and closed with recovery.

While the mean inulin clearance is numerically greater after compensation in four of the six patients, this is statistically significant only in Case 6 (J. Y.). The phenol red clearance as an indication of renal blood flow is numerically greater in all cases, and the difference is significant in all but Case 3 (J. F.). The urea clearance is also increased after cardiac compensation, but the significance is difficult to estimate since the majority of observations during decompensation were made with urine flows of less than 2 cc. per minute (standard clearance), while many of those after compensation represent maximum clearances.

If the phenol red clearance may be regarded as a measure of renal blood flow, it is possible to calculate any change in the fraction of the cardiac output which goes to the kidneys with restoration of compensation. In Case 1, the phenol red clearance represents 6.4 per cent of the cardiac output during cardiac failure, and 6.1 per cent after restoration of compensation. In Case 2, the values are 2.6 and 7.2 per cent; in Case 3, 6.0 and 5.5 per cent; in Case 4, 5.7 and 6.3 per cent; in Case 5, 5.7 and 8.0 per cent; and in Case 6, 4.1 and 6.1 per cent, giving an average of 5.1 per cent during failure and 6.5 per cent after recovery. Thus, the phenol red clearance represents about the same fraction of the cardiac output both during failure and after improvement. If the usual ratio between phenol red and diodrast clearance found by Smith be assumed, and minimal renal blood flow calculated from this and hematocrit, the conclusion is unchanged: renal blood flow both during failure and after improvement represents about 20 per cent of the estimated cardiac

output. This corresponds with the average of 29 per cent found by Smith, Goldring, and Chasis (38) for normal persons receiving a constant intravenous infusion.

The constancy of the inulin clearance with changes in renal blood flow implies changes in glomerular capillary pressure and in the fraction of the plasma filtered. An increase in glomerular capillary pressure during decompensation might be brought about by an increased tone (constriction) of the efferent vessel, dilatation of afferent vessel, or by increased pressure in the renal vein. The existence of the former mechanism of control of capillary pressure was described by Richards and Plant (40) and has been assumed by Chasis, Ranges, Goldring and Smith (41) to be an important mechanism in regulating the renal circulation. Smith finds the inulin clearance constant in normal kidneys both during adrenalin ischemia and pyrexial hyperemia, in spite of changes in the diodrast clearance which is used as a measure of renal blood flow. Constriction of efferent vessel diminishes renal flow. If this mechanism is responsible for the constant inulin clearance, it implies that were such constriction not present a greater fraction of the cardiac output would go to the kidneys in the decompensated patient than in the normal patient. No mechanism to account for such a hypothesis is apparent. If, however, only efferent vessels take part in the generalized increase in peripheral resistance of cardiac failure, the data could be accounted for on such a basis. Afferent vessels, however, respond in the usual way to central and chemical constriction or stimuli. Similarly, dilatation of afferent vessels, other conditions remaining the same, gives an increased renal flow (42) and glomerular pressure. The constant percentage of the cardiac output represented by the phenol red clearance would seem to make this mechanism unlikely. The higher diastolic pressure and calculated peripheral resistance during decompensation are probably an indication of arteriolar constriction, so that if afferent arterioles were dilated, their state would have to be assumed to differ from that of other small arteries in the body.

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While the mean inulin clearance is numerically greater after compensation in four of the six patients, this is statistically significant only in Case 6 (J. Y.). The phenol red clearance as an indication of renal blood flow is numerically greater in all cases, and the difference is significant in all but Case 3 (J. F.). The urea clearance is also increased after cardiac compensation, but the significance is difficult to estimate since the majority of observations during decompensation were made with urine flows of less than 2 cc. per minute (standard clearance), while many of those after compensation represent maximum clearances.

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In hyperthyroidism (Table VII), both phenol red and inulin clearances represent about the same fraction of the cardiac output before and after thyroidectomy, and the phenol red/inulin ratio is

unchanged, so that here it seems unnecessary to postulate any changes in intrarenal vascular adjustments.

Hence, it seems that the simplest explanation of the data on the cardiac patients is that renal blood flow is reduced in proportion to the reduction in cardiac output, and that the high venous pressure is responsible for an abnormally high

TABLE VII

Changes in renal function before and after thyroidectomy for hyperthyroidism

Case	Inulin clearance	Phenol red clearance	Phenol red/Inulin	Urea clearance	Blood urea nitrogen
				percent	
1.	183 128	367 322	2.0 2.5	144 107	7.6 4.4
2.	117 110	392 345	3.3 3.1	80 80	17.3 9.9
3.	167 120	572 395	3.4 3.3	97 80	15.0

glomerular capillary pressure with a consequent increase in the fraction of the plasma water which is filtered.

It should be pointed out that the decrease in kidney function accompanying heart failure is not associated with any decrease in concentrating power, so that there is no evidence that the passive congestion and diminished blood flow have impaired tubular function. Since these cells are carrying out their usual function normally, it may be presumed that the ability to secrete phenol red is likewise unimpaired. The mean phenol red/inulin clearance ratio in these patients after recovery was 3.0, which is slightly lower than the average of 3.3 given by Smith.

SUMMARY

Estimations of cardiac output, volume of blood and interstitial fluid, concentration and total amount of plasma proteins, and of inulin, phenol red and urea clearances were made on six patients with congestive heart failure and again after restoration of compensation by digitalis and diuretics.

Cardiac output and stroke volume increased with improvement in all cases. The mean minute output during failure was 31 per cent less than after improvement. Effective peripheral resistance decreased coincidentally with improvement.

Blood and serum volumes also decreased with recovery, the mean change in blood volume being 1.15 liters or 25 per cent, and in serum volume 0.92 liter or 35 per cent of the volumes during failure. In spite of a marked decrease in the volume of intercellular fluid, the mean value after restoration of compensation was 32 per cent of total body weight, or almost 50 per cent above normal.

While the concentration of serum proteins, particularly of the globulin fraction, increased with clinical improvement, the total amount of circulating protein decreased, due almost entirely to a decrease in serum albumin.

Inulin clearance showed no significant change with improvement, while phenol red clearance increased to approximately the same degree as the cardiac output.

PROTOCOLS

Case 1, F. T., Hospital Number 201-250. A 66-year-old Italian laborer entered the hospital on November 25, 1939, because of dyspnea and edema of legs. He had had mild symptoms of failure for two years. Two months prior to admission, dyspnea became severe and he developed generalized edema. He had taken no digitalis. Physical examination showed a large heart, a large tender liver, and edema of both extremities with a large abdomen without demonstrable fluid. Pulse was 110 and blood pressure was 240/140. Vital capacity was 1,200 cc. After the data were obtained, he was digitalized, responding with a fair diuresis and relief of symptoms. On his sixth hospital day he developed pneumonia, which responded well to sulfapyridine, and he was discharged symptomatically improved and edema free after the second set of determinations were performed twenty-eight days later. Blood pressure on discharge was 180/110. Diagnosis: Hypertensive heart disease and cardiac decompensation.

Case 2, P. K., Hospital Number 201-932. A 71-year-old white male entered the hospital on December 27, 1939, because of dyspnea and edema of the legs. Eight years previously he had had mild symptoms of failure, and three years previously he had had symptoms suggestive of a myocardial infarct. He had received digitalis intermittently with relief of symptoms, but two months before admission edema became more noticeable and had recently become massive. He had taken no digitalis within three weeks. On physical examination the heart was enlarged, there were pleural effusions at both lung bases, the abdomen contained fluid, and massive pitting edema extended from the ankles to the scrotum. Pulse was 90 and blood pressure 160/90. Vital capacity was 2,000 cc. The patient was digitalized and obtained symptomatic improvement. On several occasions salyrgan administration

resulted in a copious diuresis. He was discharged edema free after twenty-six days in the hospital. Blood pressure on discharge was 140/80. Diagnosis: Arteriosclerotic heart disease with cardiac decompensation.

Case 3, J. F., Hospital Number 202-176. A 72-year-old white man was admitted on January 8, 1940, complaining of sudden onset of dyspnea and edema two weeks previously. Physical examination showed marked orthopnea, right hydrothorax, and numerous râles over the left lung. The heart was enlarged 12 cm. to the left of the mid-sternal line. A harsh systolic murmur was present over the entire precordium but was best heard over the second interspace just to the right of the sternum. Blood pressure was 165/95. The liver was palpable 5 cm. below the costal margin and was tender. There was pitting edema to the level of the sacrum. Peripheral arteriosclerosis was marked. He was treated with digitalis and mercurial diuretics with marked improvement and was discharged on his thirty-first hospital day with a blood pressure of 150/95 and free from demonstrable edema. Diagnosis: Arteriosclerotic heart disease with hypertension and cardiac decompensation.

Case 4, J. G., Hospital Number 210-731. A 52-year-old white man was admitted on January 8, 1941, with the history of ankle edema and dyspnea on exertion of three months' duration. There was slight arteriovenous notching of the vessels of the fundi. Moist râles were present over the bases of both lungs. The heart was enlarged; no murmurs were heard; blood pressure was 210/118. The liver was palpable three fingers' breadth below the costal margin. Pitting edema was present to the level of the sacrum. He was treated with digitalis and mercurial diuretics and showed a satisfactory response. He was discharged on his forty-second hospital day with a blood pressure of 160/90 and free from demonstrable edema. Diagnosis: Hypertensive cardiovascular disease with cardiac decompensation.

Case 5, M. A., Hospital Number 205-085. A 70-year-old white man was admitted on May 20, 1940, with the complaint of swelling of the legs and abdomen of three months' duration. Physical examination showed cyanosis, arteriovenous notching of the retinal vessels, moist râles at the left lung base. The heart was enlarged 14 cm. to the left of the mid-sternal line. There was a protodiastolic gallop rhythm. No murmurs were heard. Blood pressure was 140/90. The liver was tender and palpable 5 cm. below the costal margin. A fluid wave was present in the abdomen. Generalized arteriosclerosis was marked. There was edema of the legs to the level of the sacrum. Satisfactory compensation was obtained with digitalis and mercurial diuretics. He was discharged on the twenty-sixth hospital day with a blood pressure of 140/90 and free from demonstrable edema. Diagnosis: Arteriosclerotic heart disease with cardiac decompensation.

Case 6, J. Y., Hospital Number 208-229. A 65-year-old white man was admitted on September 18, 1940, with the

chief complaints of dyspnea on exertion and swelling of the legs of four months' duration. Physical examination showed moderate cyanosis, distended neck veins, and râles over both lower lobes of the lungs. The heart was enlarged 13 cm. to the left of the mid-sternal line, and 5 cm. to the right. A systolic murmur was present at the apex. Blood pressure was 142/100. The liver was enlarged, extending 8 cm. below the costal margin and was tender. There was pitting edema of the legs to the level of the sacrum. The peripheral arteries were markedly sclerotic. Satisfactory compensation was obtained with digitalis and mercurial diuretics and he was discharged on the thirty-third hospital day with a blood pressure of 190/60 and free from demonstrable edema. Diagnosis: Arteriosclerotic heart disease with cardiac decompensation.

BIBLIOGRAPHY

1. Stewart, H. J., and Cohn, A. E., Studies on the effect of the action of digitalis on the output of blood from the heart. *J. Clin. Invest.*, 1932, 11, 917.
2. Starr, I., Jr., and Gamble, C. J., Cardiac output in common clinical conditions, and the diagnosis of myocardial insufficiency by cardiac output methods. *Ann. Int. Med.*, 1935, 9, 569.
3. MacKenzie, Sir James, *Diseases of the Heart*. Oxford Univ. Press, London, 1925.
4. Lewis, Sir Thomas, *Diseases of the Heart*. Mac-Millan Company, New York, 1933.
5. Friedman, B., Clark, G., Resnik, H., and Harrison, T. R., Effect of digitalis on the cardiac output of persons with congestive heart failure. *Arch. Int. Med.*, 1935, 56, 710.
6. Harrison, T. R., *Failure of the Circulation*. Williams and Wilkins, Baltimore, 1939, 2nd ed.
7. Fishberg, A. M., *Heart Failure*. Lea and Febiger, Philadelphia, 1937.
8. Richards, A. N., and Plant, O. H., Urine formation in the perfused kidney. The influence of alterations in renal blood pressure on the amount and composition of urine. *Am. J. Physiol.*, 1922, 59, 144.
9. Grollman, A., *The Cardiac Output of Man in Health and Disease*. C. C. Thomas, Baltimore, 1932.
10. Bazett, H. C., Cotton, F. S., LaPlace, L. B., and Scott, J. C., The calculation of cardiac output and effective peripheral resistance from blood pressure measurements with an appendix on the size of the aorta in man. *Am. J. Physiol.*, 1935, 113, 312.
11. Bazett, H. C., Scott, J. C., Maxfield, M. E., and Blithe, M. D., Calculation of cardiac output from blood pressure measurements before and after meals. *Am. J. Physiol.*, 1936, 116, 551.
12. Whittaker, R. F., and Winton, F. R., The apparent viscosity of blood flowing in an isolated hindlimb of the dog, and its variations in corpuscular content. *J. Physiol.*, 1933, 78, 339.
13. Gregg, D. E., Eckstein, R. W., and Fineberg, M. H., Pressure pulses and blood pressure values in unanesthetized dogs. *Am. J. Physiol.*, 1937, 118, 399.

13. Lyons, R. H., Kennedy, J. A., and Burwell, C. S., The measurement of venous pressure by the direct method. *Am. Heart J.*, 1938, 16, 675.
14. Wiggers, C. J., The dynamics of hypertension. *Am. Heart J.*, 1938, 16, 515.
15. Bazett, H. C., Blood volume and cardiovascular adjustments, *Am. Heart J.*, 1941, 21, 423.
16. Starr, I., Rôle of the "static blood pressure" in abnormal increments of venous pressure, especially in heart failure. *Am. J. Med. Sc.*, 1940, 199, 1.
17. Payne, S. A., and Peters, J. P., The plasma proteins in relation to blood hydration. VIII. Serum proteins in heart disease. *J. Clin. Invest.*, 1932, 11, 103.
18. Cope, C. L., The osmotic pressure of the blood proteins in nephritis. *Quart. J. Med.*, 1928, 22, 91.
19. Epstein, A. A., A contribution to the study of the chemistry of blood serum. *J. Exper. Med.*, 1912, 16, 719.
20. Thomson, W. R. A., The plasma proteins and cardiac oedema. *Quart. J. Med.*, 1934, 3, 587.
21. Hand, H. M., Concentration of serum protein in different types of edema. *Arch. Int. Med.*, 1934, 54, 215.
22. Rowe, A. H., Refractometric studies of serum proteins in nephritis, cardiac decompensation, diabetes, anemia, and other chronic diseases. *Arch. Int. Med.*, 1917, 19, 354.
23. Ellis, L. B., Plasma protein deficiency in patients with cardiac edema. *Med. Clin. North Amer.*, 1933, 16, 943.
24. Iversen, P. U., and Nakazawa, F., Über die Biochemie Des Filtrationsödems. *Biochem. Ztschr.*, 1927, 191, 307.
25. Unpublished data from the laboratory of Dr. J. P. Peters, New Haven Hospital.
26. Loeb, R. F., Plasma proteins in health and disease. *New England J. Med.*, 1941, 224, 980.
27. Gibson, J. G., 2nd, and Evans, W. A., Jr., Clinical studies in blood volume. III. Changes in blood volume, venous pressure, and blood velocity rate in chronic congestive heart failure. *J. Clin. Invest.*, 1937, 16, 851.
28. Gibson, J. G., 2nd, and Evans, W. A., Jr., Clinical studies in blood volume. I. Clinical application of a method employing the azo dye "Evans Blue" and the spectrophotometer. *J. Clin. Invest.*, 1937, 16, 301.
29. Laviertes, P. H., Bourdillon, J., and Klinghoffer, K. A., The volume of the extracellular fluids of the body. *J. Clin. Invest.*, 1936, 15, 261.
30. Robinson, H. W., Price, J. W., and Hogden, C. G., The estimation of albumin and globulin in blood serum. III. The precipitation of globulin at twenty-five degrees by sodium sulfate. *J. Biol. Chem.*, 1938, 126, 213.
Robinson, H. W., Price, J. W., and Hogden, C. G., The estimation of albumin and globulin in blood serum. II. Separation of fractions by centrifugation with the angle centrifuge. *J. Biol. Chem.*, 1938, 126, 207.
31. Landis, E. M., The passage of fluid through the capillary wall. *Am. J. M. Sc.*, 1937, 193, 297.
32. Madden, S. C., and Whipple, G. H., Plasma proteins: Their source, production, and utilization. *Physiol. Rev.*, 1940, 20, 194.
33. Stewart, J. D., and Rourke, G. M., Changes in blood and interstitial fluid resulting from surgical operation and ether anaesthesia. *J. Clin. Invest.*, 1938, 17, 413.
34. Corcoran, A. C., and Page, I. H., Application of diphenylamine in the determination of levulose in biological media. *J. Biol. Chem.*, 1939, 127, 601.
35. Van Slyke, D. D., Determination of urea by gasometric measurement of the carbon dioxide formed by the action of urease. *J. Biol. Chem.*, 1927, 73, 695.
36. Van Slyke, D. D., and Kugel, V. H., Improvements in manometric micro-Kjeldahl and blood urea methods. *J. Biol. Chem.*, 1933, 102, 489.
37. Smith, H. W., *The Physiology of the Kidney*. Oxford University Press, New York, 1937.
38. Smith, H. W., Goldring, W., and Chasis, H., The measurement of the tubular excretory mass, effective blood flow and filtration rate in the normal human kidney. *J. Clin. Invest.*, 1938, 17, 263.
39. Spanner, R., Der Abkürzungskreislauf der menschlichen Niere. *Klin. Wchnschr.*, 1937, 16, 1421.
40. Richards, A. N., and Plant, O. H., Urine formation in the perfused kidney. The influence of adrenalin on the volume of perfused kidney. *Am. J. Physiol.*, 1922, 59, 184.
41. Chasis, H., Ranges, H. A., Goldring, W., and Smith, H. W., The control of renal blood flow and glomerular filtration in normal man. *J. Clin. Invest.*, 1938, 17, 683.
42. Richards, A. N., The nature and mode of regulation of glomerular function. *Am. J. M. Sc.*, 1925, 170, 781.

THE EFFECT OF FOREIGN SURFACES ON BLOOD COAGULATION ^{1, 2}

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In the United States, the two-stage theory of blood coagulation is widely accepted. This theory recently reviewed by Quick (1) and Eagle (2) suggests that in the first stage prothrombin, calcium ion and thromboplastin interact to form thrombin. Thrombin, in the second stage, converts fibrinogen to fibrin. Plasma thromboplastin has been considered to be of platelet origin. The phenomenon of the initiation of coagulation when blood is shed has been considered by many investigators to be due to the disintegration of platelets with the release of thromboplastin.

It has been known for many years that the coagulation time of blood taken in paraffin tubes is much longer than that of blood taken in glass tubes. The explanation usually offered for this phenomenon is that the glass, acting as a foreign surface, destroys the platelets at a more rapid rate than occurs in the presence of paraffin. The increased amount of thromboplastin so liberated is then considered to be responsible for the shorter coagulation time in glass vessels. Nolf (3) has criticized this explanation. He states that his experimental data indicate that cell- and platelet-free plasma already contains all of the factors necessary for blood coagulation. He believes that the initiation of coagulation is the result of the direct modification of one or more of the constituents of cell-free plasma by contact with a foreign surface such as glass.

In view of this difference of opinion, it seemed advisable to reinvestigate the rôle which foreign surfaces play in the blood coagulation reaction.

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The present communication reports certain observations on the effects of four such foreign surfaces. The materials investigated were glass, paraffin, collodion, and a synthetic plastic "Lusteroid."³

METHODS

Coagulation times were determined on venous blood by Pohle and Taylor's (5) modification of the method of Lee and White (4). Platelet counts were made by a direct counting method (6). By this method "platelet" counts below 15,000 per cu. mm. are without significance since cell-free Berkefeld filtered plasmas will give such values due to the presence of non-specific refractile bodies. Venepunctures were made with an oiled syringe and needle, and *only when the vein was entered on the initial attempt and with minimum trauma* was the blood used for these investigations. Plasma globulin substance was made from cell-free plasma, as previously described (7), with such modifications as are given below. For the sake of brevity one typical experiment on each phase of the investigation is given. In all instances however, the results were repeatedly duplicated.

EXPERIMENTAL

The effect of foreign surfaces on the coagulation of platelet-free plasma

Experiments of this type in the past have been criticized on the basis that either specialized plasmas, such as bird plasma, were employed or that a non-physiological anticoagulant was added which might modify the system under investigation. Therefore, the initial experiments were performed on *normal human blood plasma to which no anticoagulant was added*. Normal human venous blood was taken with an oiled syringe and transferred to a Lusteroid tube. In one typical experiment the platelet count on the blood was initially 300,000 per cu. mm. The tube and contents were centrifuged in an angle head centrifuge at 4,200 r.p.m. for 10 minutes and the resultant crystal clear plasma removed by a collodion coated pipette to a

³ Obtainable in the form of centrifuge tubes from the International Equipment Company, Boston, Mass.

second scrupulously clean and dry Lusteroid tube. The "platelet count" on this plasma was 8,000 per cu. mm., or within the blank for the method. Two ml. of the plasma were transferred with collodion pipettes to tubes of each of the four materials under investigation.

TABLE I

The effect of foreign surfaces on the coagulation time of platelet-free plasma prepared without any anticoagulant

2 ml. plasma placed in	Coagulation time
	minutes
Glass	11
Collodion	64
Paraffin	44
"Lusteroid"	49

The data shown in Table I indicate that coagulation occurred in the glass tube in 11 minutes, which is within the normal time for this subject. In tubes of the other three materials the coagulation time was markedly delayed. Since the plasma used was platelet-free, the effect of the glass surface could not reasonably be attributed to the lysis of platelets.

In order to allow for more extensive manipulation of plasma in determining the site of action of foreign surfaces, the remainder of the investigation was carried out on plasma from blood containing sodium citrate in a final concentration of 0.25 per cent as an anticoagulant. The concept that the presence of such an anticoagulant modifies the behavior of plasma toward foreign surfaces must be abandoned since the results of the following experiments, in which citrated plasma was used, are entirely comparable to the results already described in which no anticoagulant was used.

The site of action of the foreign surface

Normal human venous blood was obtained in the manner suggested and transferred to Lusteroid tubes containing the requisite volume of 2.5 per cent sodium citrate. *Platelet-rich plasma* was obtained by slowly centrifuging the blood at about 1500 r.p.m. for 10 minutes. Samples of the slightly opalescent plasma were placed in tubes of the four materials and kept at room temperature for one hour. Platelet counts were made and 2 ml. samples were transferred to Lusteroid tubes and the plasmas recalcified. The results of a

typical experiment are shown in Table II. The data show that no significant change in the platelet counts occurred following the exposure of the platelet-rich plasma to the four foreign surfaces for one hour. Preliminary experiments have shown that platelet destruction can be followed readily by the direct counting method. It would appear, therefore, that in the presence of these four foreign surfaces *there was no increased lysis of platelets*. However, in spite of this observation, the coagulation time of the plasma which had been exposed to glass for one hour prior to its recalcification was markedly less than that obtaining for the three samples of plasma which had been exposed for the same length of time to the other three surfaces.

TABLE II

*The effect of foreign surfaces on the platelet count and the coagulation time of recalcified * citrated platelet-rich normal human plasma*

Plasma incubated one hour in	Platelet count	Coagulation time in "Lusteroid"
	per mm. ³	minutes
Glass	412,000	6
Collodion	414,000	18
Paraffin	412,000	20
"Lusteroid"	432,000	20

* 2 ml. plasma + 0.4 ml. 0.5 per cent CaCl₂.

To further rule out lysis of the intact platelet as the responsible agent in foreign surface effect, the observations were repeated, except that the normal citrated plasma was rendered *free from platelets* by centrifuging at 4,200 r.p.m. for 15 minutes before transferring to the tubes made of the four materials under investigation. The data of one typical observation (Table III) show that, when 2 ml. of such platelet-free plasmas are recalcified in Lusteroid tubes, the one exposed to glass for one hour coagulates in 11 minutes, which is

TABLE III

The effect of foreign surfaces on the platelet count and the coagulation time of recalcified citrated platelet-free normal human plasma

Plasma incubated one hour in	Platelet count	Coagulation time in "Lusteroid"
	per mm. ³	minutes
Glass	12,000	11
Collodion	8,000	30
Paraffin	4,000	39
"Lusteroid"	10,000	38

within the range usually encountered for the coagulation time of normal citrated plasma after recalcification in glass tubes. Those samples of plasma exposed to the other three surfaces for the same length of time had markedly prolonged coagulation times.

It has recently been reported that there exists in cell-free citrated normal human plasma a factor, independent of prothrombin and fibrinogen (8, 9), capable of reducing the coagulation time of hemophilic blood *in vivo* and *in vitro*. The effect of foreign surfaces on this plasma factor was studied. Normal citrated platelet-rich plasma was prepared without exposure to glass and transferred to tubes of glass, collodion, paraffin and Lusteroid. The plasmas were permitted to remain in contact with these surfaces for one hour at room temperature. One-tenth of a milliliter of each of these samples of plasma was introduced into Lusteroid tubes and 2 ml. of hemophilic blood, obtained without exposure to glass, were added as soon as possible after the venepuncture. The results of a typical experiment are shown in Table IV. It will be

TABLE IV

The effect of foreign surfaces on the platelet count and the clot-promoting activity for hemophilic blood of citrated platelet-rich normal human plasma

Plasma incubated one hour in	Platelet count	Coagulation time in "Lusteroid" of 2 ml. hemophilic blood +0.1 ml. plasma
	per mm. ³	minutes
Glass	412,000	14
Collodion	414,000	41
Paraffin	412,000	40
"Lusteroid"	432,000	43
Control: No plasma added		300

observed that the normal plasma exposed to glass for one hour had a much greater clot-promoting power for hemophilic blood than had samples of the same plasma which had been exposed to the other three surfaces for the same length of time. The control coagulation time shown in Table IV is that obtained in Lusteroid tubes. The data also indicate that no lysis of platelets occurred during the one-hour exposure to the foreign surfaces.

The observations recorded above were repeated, using platelet-free plasma. For this purpose the normal citrated human plasma was centrifuged at 4,200 r.p.m. for 15 minutes. At the end of this

procedure the "platelet counts" were within the blank for the method and the plasma was crystal clear. Portions of this plasma were again exposed to contact with the four foreign surfaces for one hour at room temperature. One tenth millimeter portions of these plasmas were tested, as described, against 2 ml. of hemophilic blood in Lusteroid tubes. The results obtained in one typical experiment (Table V) show that, although the citrated

TABLE V

The effect of foreign surfaces on the platelet count and the clot-promoting activity for hemophilic blood of citrated platelet-free normal human plasma

Plasma incubated one hour in	Platelet count	Coagulation time in "Lusteroid" of 2 ml. hemophilic blood +0.1 ml. plasma
	per mm. ³	minutes
Glass	12,000	25
Collodion	8,000	88
Paraffin	4,000	95
"Lusteroid"	10,000	90
Control: No plasma added		300

normal plasma was platelet-free, nevertheless that portion exposed to glass had a far greater clot-promoting activity for hemophilic blood than did those portions exposed to the other foreign surfaces.

It would appear from the data thus far presented that variation in the rate of lysis of the platelets could not be the essential explanation of the foreign surface effect and that the fraction of normal cell-free plasma which is involved in the foreign surface effect is the same fraction which, when added to hemophilic blood, both *in vivo* and *in vitro*, results in a sharp reduction in its coagulation time. This point was further investigated.

The clot-promoting activity for hemophilic blood is associated with the euglobulin fraction of cell-free plasma (8, 9). This fraction has been called in publications from this laboratory "globulin substance" and by Howell (10) "plasma thromboplastin." Observations were made concerning the effect of foreign surfaces on the clot-promoting activity of globulin substance prepared by acid precipitation of diluted, citrated, cell-free normal human plasma in vessels of glass, collodion and paraffin. Lusteroid vessels other than tubes are not at present available so that this material could not be included in the observations.

The acid precipitated globulin substance from each of the three vessels was dissolved in 0.9 per cent sodium chloride solution, equal in volume to the plasma from which it was derived. One tenth ml. of these saline solutions was pipetted into the bottom of Lusteroid tubes and 2 ml. of hemophilic blood preserved from glass contact were added. The results (Table VI) show that

TABLE VI

The effect of foreign surfaces on the clot-promoting activity for hemophilic blood of "globulin substance" prepared from citrated normal human plasma

"Globulin substance" prepared in	Coagulation time in "Lusteroid" of 2 ml. hemophilic blood +0.1 ml. "globulin substance"
	<i>minutes</i>
Glass	10
Collodion	50
Paraffin	62
Control	360

the globulin substance from plasma exposed to glass throughout the procedure had significantly more clot-promoting activity for hemophilic blood than that prepared from plasma exposed to surfaces of the other materials.

DISCUSSION

The foregoing observations show that the effect of foreign surfaces on blood coagulation are essentially independent of the intact platelet. These studies indicate that the site of action of such surfaces is dependent upon a factor which is present in normal cell-free plasma, independent of prothrombin and fibrinogen (9), associated with the euglobulin fraction of the proteins (8) and concerned with the clot-promoting activity of plasma for hemophilic blood. That this globulin substance might be derived from damaged platelets cannot be excluded by the present observations. However, it is present in cell-free plasma, and foreign surfaces have entirely similar effects on platelet-free and platelet-rich plasma. The data presented clearly indicate that the effect of foreign surfaces is not accompanied by any increased lysis of the platelets prior to clot formation, as heretofore assumed.

Exposure to a glass surface had more effect in "activating" the factor involved than did surfaces of paraffin, collodion or Lusteroid. The term "activating" is applied to the effect of glass rather

than explaining the effect of collodion, paraffin and Lusteroid as due to an "inhibition" of the plasma factor involved, because plasma placed first in glass vessels and then transferred to tubes of the other foreign surfaces behaves as plasma exposed to glass alone.

The exact mechanism of the foreign surface effect can at present be only hypothecated. A possible hint of the type of reaction involved lies in the work of Gortner and Briggs (11) who showed by streaming potential methods that the charge on a glass surface in contact with water is considerably greater than that on a paraffin surface. These authors have also suggested that a positively charged substance in blood may be adsorbed by a negative surface such as that presented by a glass interface. These demonstrations make it inviting to suggest that the foreign surface effect is due to the physico-chemical modification of one or more of the constituents of cell-free plasma.

The data of the present communication give experimental confirmation to some of the concepts of the initiation of coagulation expressed by Nolf (3). They also invite the speculation that the so-called "physiological anticoagulant" sought by many investigators as an explanation for the fluidity of circulating blood may be found in the probability that the vascular endothelium may resemble such surfaces as collodion, paraffin, and "Lusteroid" in its behavior toward some non-cellular plasma constituent.

SUMMARY

1. The effects of four foreign surfaces—glass, paraffin, collodion and Lusteroid—on the initiation of blood coagulation were studied.
2. Glass was shown to have the greatest effect on initiating coagulation.
3. No lysis of the platelets was found following exposure of platelet-rich plasma for one hour to any of the foreign surfaces studied.
4. The data suggest that the foreign surface acts by some modification of some constituent of cell-free plasma, probably by physico-chemical change.
5. It is suggested that at least one of the plasma factors modified is the plasma euglobulin fraction known as "globulin substance" and called by Howell "plasma thromboplastin."

BIBLIOGRAPHY

1. Quick, A. J., A classification of hemorrhagic diseases due to defects in the coagulation mechanism of the blood. *Am. J. M. Sc.*, 1940, 199, 118.
2. Eagle, H., The present status of the blood coagulation problem. A symposium on the blood and blood forming organs. The University of Wisconsin Press, Madison, Wisconsin, 1939, 242.
3. Nolf, P., Eine neue Theorie der Blutgerinnung. *Ergebn. d. inn. Med. u. Kinderh.*, 1912, 59, 275.
4. Lee, R. I., and White, P. D., Clinical study of the coagulation of blood. *Am. J. M. Sc.*, 1913, 145, 495.
5. Pohle, F. J., and Taylor, F. H. L., The coagulation defect in hemophilia. The effect in hemophilia of intramuscular administration of a globulin substance derived from normal human plasma. *J. Clin. Invest.*, 1937, 16, 741.
6. Pohle, F. J., The blood platelet count in relation to the menstrual cycle in normal women. *Am. J. M. Sc.*, 1939, 197, 40.
7. Patek, A. J., and Taylor, F. H. L., Hemophilia. II. Some properties of a substance obtained from normal human plasma effective in accelerating the coagulation of hemophilic blood. *J. Clin. Invest.*, 1937, 16, 113.
8. Lozner, E. L., and Taylor, F. H. L., The coagulation defect in hemophilia: Studies on the clot promoting activity associated with plasma euglobulin in hemophilia. *J. Clin. Invest.*, 1939, 18, 821.
9. Lozner, E. L., Kark, R., and Taylor, F. H. L., The coagulation defect in hemophilia: The clot promoting activity in hemophilia of Berkefelded normal human plasma free from fibrinogen and prothrombin. *J. Clin. Invest.*, 1939, 18, 603.
10. Howell, W. H., Hemophilia, the Wesley M. Carpenter Lecture. *Bull. N. Y. Acad. Med.*, 1939, 15, 3.
11. Gortner, R. A., and Briggs, D. R., Glass surfaces vs. paraffin surfaces in blood clotting phenomena—hypothesis. *Proc. Soc. Exper. Biol. and Med.*, 1928, 25, 820.

THE URINARY EXCRETION OF THIAMINE AS AN INDEX OF THE NUTRITIONAL LEVEL: ASSESSMENT OF THE VALUE OF A TEST DOSE

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Harris and Leong (1), in 1936, suggested that the state of vitamin B₁ deficiency in man could be detected by measurement of the amount of the vitamin excreted in the urine in the twenty-four-hour period following a single oral test dose of 900 micrograms of thiamine chloride, and since then many other procedures have been suggested for the detection of vitamin B₁ deficiency by means of a test dose (2 to 8). Oral and parenteral routes have been used for administration of test doses varying from 1 to 10 mgm. of thiamine chloride and various criteria have been set up for estimation of the state of the subject's nutrition with respect to vitamin B₁.

The value of a laboratory test for vitamin B₁ deficiency lies largely in its application to the detection of those cases in which a chronic mild deficiency may have existed for some time but in which cleancut symptoms or signs of deficiency are not evident. The urinary excretion of thiamine under various conditions has been used as a suggestive index of the state of thiamine nutrition but, unfortunately, most of the control tests reported in the literature have been made on persons partaking of their customary diets of unknown or unspecified vitamin B₁ content, or on hospital patients. We believe that it is necessary to determine the response to the test of individuals maintained on various levels of thiamine intake for varying periods of time in order to assess the validity of the test. Only in this way can the response be correlated satisfactorily with the nutritional history and clinical state.

We are prompted to report our experience with test-dose procedures because we have had the opportunity to study the responses of fifteen female subjects maintained on rigidly controlled diets of known thiamine content for intervals varying from thirteen weeks to five months. Factors of the vitamin B complex other than thiamine were supplied in purified form or in the form of

autoclaved yeast; therefore, the dietary restriction was limited as nearly as possible to thiamine alone. Since the duration and degree of deficiency were known accurately, we believe that the value of test-dose procedures or of the ordinary twenty-four-hour excretion of thiamine as a laboratory aid can be accurately assessed.

EXPERIMENTAL

The present study is part of a larger one on induced thiamine deficiency in human subjects. The selection of subjects and the diets utilized in this study have been described in previous reports (9, 10, 11). Two groups of four women each, who agreed to serve as subjects for this study, were fed a diet which contained not more than 150 micrograms per day of thiamine, and a third group of eleven women were fed a diet which furnished 400 to 450 micrograms of thiamine per day.

Test-dose procedure. There is no agreement as to the amount of thiamine that should be used for a test dose or as to the mode of administration. Intramuscular injection was chosen as the mode of administration since it insured complete and rapid absorption. A test dose of 1 mgm. (1000 micrograms) of thiamine chloride was chosen since it was sufficiently large for the purpose but not large enough to flood the system when rapidly absorbed. The results confirm the adequacy of this procedure.

Collection of urine. Urine was collected in bottles which contained 15 cc. of glacial acetic acid, an amount sufficient to bring the pH of a twenty-four-hour specimen to about 4. Specimens were collected during the twenty-four hours just preceding and again immediately following the injection of the test dose. The thiamine content was determined by the method of Hennessy and Cerecedo (12), revised by Hennessy (13).

RESULTS

Table I shows the values of thiamine excretion in two groups of four subjects who were maintained on a diet which contained not more than 150 micrograms of thiamine per day. The first group (Subjects 1 to 4) received the test dose after 155 days and again after 169 days at this intake level. Symptoms of severe deficiency of

thiamine were evident on both occasions. The results of the test were essentially the same in both instances. The excretions of thiamine in the twenty-four hours preceding the test were 11 to 26 micrograms.¹ In the twenty-four hours following the injection of the test dose of 1000 micrograms, the amounts of thiamine (extra thiamine) excreted over and above those in the preceding twenty-four hours were 2 to 6 per cent of the amount injected. Following a subsequent period of seventeen days during which 10 mgm. of thiamine chloride and 10 grams of brewers' yeast were given as daily supplements to an unrestricted diet, the intake of thiamine was again restricted to 850 micrograms for seventeen days. On the last three days of this period the average (pooled specimens) twenty-four-hour excretions of thiamine were 124 to 256 micrograms and on the eighteenth day the extra excretions after the test dose were 15 to 45 per cent of the dose.

The second group of subjects (5, 6, 8 and 9) received the test dose after they had consumed the diet containing 150 micrograms of thiamine for eighty-eight days, at which time symptoms of severe deficiency of thiamine were evident. The results were similar to those obtained in the first group. After a thirty-three-day period of unrestricted diet with supplements of brewers' yeast and thiamine chloride (15 mgm. for eight days, then 5 mgm. for twenty-five days) the intake of thiamine was restricted to 850 micrograms for five days. On the fifth day the excretions were 254 to 400 micrograms and on the sixth day 23 to 26 per cent of the test dose was excreted in the urine. The intake of thiamine was further restricted to 150 micrograms for five days. The excretions during twenty-four hours fell to values between 35 and 75 micrograms and on the fifth day the extra excretions after the test dose were 12 to 17 per cent of the dose.

These results indicate a rapid loss of the thiamine stored during the period of excessive intake. The values of 254 to 400 micrograms are much higher than the values that would normally be found for the thiamine excretion when the intake is 850 micrograms. After restriction of the intake to 150 micrograms the values of 35, 41, 45, and

75 micrograms for the ordinary excretion are definitely within the range indicative of a deficient intake of thiamine. Although the recoveries of 12 to 17 per cent of the test dose are also subnormal, they reflect a better state of nutrition with respect to thiamine than the values for the ordinary excretion.

Melnick, Field and Robinson (15) also have observed elevated values for the excretion of thiamine for some days after ingestion of large amounts of thiamine chloride. They recommended that two weeks be allowed to elapse between termination of the excessive intake of thiamine and a study of the excretion. It will be shown later that evidence of storage of thiamine may be found in the ordinary excretion and in the response to the test dose as long as four weeks after restriction of the intake to 400 to 600 micrograms.

The third group of eleven subjects (5, 6 and 8 to 16; Table II), after a preliminary control period with an intake of 850 micrograms of thiamine daily, were fed a diet containing 450 micrograms of thiamine in order to study the effects of a chronic deficiency as compared with the acute deficiency produced in earlier studies. The low excretions of thiamine during the control period by Subjects 8, 9, 10 and 15 indicate that a daily intake of 850 micrograms of thiamine was insufficient for these subjects at this time. Subjects 5, 6, 8, 9 and 10 were used in the previous study in which the thiamine intake was restricted to 150 micrograms. At the beginning of the previous study the excretions of thiamine indicated that an intake of 850 micrograms of thiamine was adequate, although the period of measured intake at this level was only six days as compared with sixteen days in this later study. A period of fifty-four days had elapsed between the two studies and during fifty days these subjects were allowed an unrestricted diet supplemented with 2.5 mgm. of thiamine chloride and 10 grams of brewers' yeast daily. Just prior to the second study the thiamine supplement was increased to 7.5 mgm. for four days. The excretions of thiamine by these subjects (5, 6, 8, 9 and 10) did not differ significantly from the excretions of the other subjects of Table II who had received only the ordinary institutional diet prior to the period of study. There is thus no indication in

¹ Values of this order, although expressed as thiamine, may be largely due to the presence of metabolic products of nicotinic acid (14).

TABLE I

Excretion of thiamine before and after a test dose when the diet furnished 150 micrograms of thiamine

Subject	Age	Weight	Height	Thiamine content of diet	Interval of intake level	Twenty-four-hour excretion before injection of 1 mgm. of thiamine	Twenty-four-hour excretion after injection of 1 mgm. of thiamine	Extra excretion after test dose	Extra excretion after test dose
	years	kgm.	cm.	micrograms	days	micrograms	micrograms	micrograms	per cent of test dose
1	29	57	163	700	*	99			
				150	155	15	75	60	6
				150	169	26	81	55	6
				>10000	17				
				850†	17	124	458	334	33
2	21	54	157	700	*	90			
				150	155	19	49	30	3
				150	169	12	44	32	3
				>10000	17				
				850†	17	124	570	446	45
3	25	46	160	700	*	90			
				150	155	11	63	52	5
				150	169	12	31	19	2
				>10000	17				
				850†	17	256	405	149	15
4	24	51	157	700	*	105			
				150	155	11	72	61	6
				150	169	21	80	59	6
				>10000	17				
				850†	17	172	473	301	30
5	23	57	162	850	6	320			
				150	68	30	79	49	5
				150	88	17	32	15	2
				> 5000	33				
				850	5	400	652	252	25
6	33	51	158	150	4	35	208	173	17
				850	6	124			
				150	88	7	69	62	6
				> 5000	33				
				850	5	385	613	228	23
8	46	48	161	150	4	41	181	140	14
				850	6	98			
				150	88	9	40	31	3
				> 5000	33				
				850	5	255	510	255	26
9	26	70	159	150	4	45	166	121	12
				850	6	68			
				150	88	9	22	13	1
				> 5000	33				
				850	5	254			
9	26	70	159	150	4	75	230	155	16

* The customary institutional diet of the subject was calculated to contain 650 to 750 micrograms of thiamine. A preliminary period of measured diet was not obtained.

† After the period of restricted intake of thiamine the diet was unrestricted and was supplemented with 10 mgm. of thiamine chloride and 10 grams of brewers' yeast daily, September 12 to 29, 1939. A controlled diet which contained 850 micrograms was instituted September 29. The test was conducted October 16, 1939.

the excretions of a store of thiamine. Unfortunately, a test dose was not given at this time.

In Table II are presented the results of the administration of the test dose twenty-eight and ninety-eight days after restriction of the intake

of thiamine to 450 micrograms. The values for the ordinary twenty-four-hour excretions on the twenty-eighth day are within the range of 10 to 33 micrograms, with an average value of 16 micrograms. The values for the ninety-eighth day

TABLE II

Excretion of thiamine before and after a test dose when the diet furnished 450 micrograms of thiamine

Subject	Age	Weight	Height	Thiamine content of diet	Interval of intake level	Twenty-four-hour excretion before injection of 1 mgm. of thiamine	Twenty-four-hour excretion after injection of 1 mgm. of thiamine	Extra excretion of thiamine after test dose	Extra excretion of thiamine after test dose
	<i>years</i>	<i>kgm.</i>	<i>cm.</i>	<i>micrograms</i>	<i>days</i>	<i>micrograms</i>	<i>micrograms</i>	<i>micrograms</i>	<i>per cent of test dose</i>
5	23	57	162	850 450 450	16 28 98	135 14 24	110 116	96 92	10 9
6	33	51	158	850 450 450	16 28 98	128 10 18	77 89	67 71	7 7
8	46	48	161	850 450 450	16 28 98	64 14 23	85 60	71 37	7 4
9	26	70	159	850 450 450	16 28 98	74 19 21	75 30	56 9	6 1
10	28	53	154	850 450	16 28	73 17	55	38	4
11	39	49	158	850 450 450	16 28 98	136 16 14	116 104	100 90	10 9
12	39	53	168	850 450 450	20 28 105	96 11 18	126 21	115 3	12 <1
13	37	85	157	850 450 450	20 28 98	100 33 23	86 63	53 40	5 4
14	45	55	166	850 450 450	20 28 98	140 19 16	95 93	76 77	8 8
15	41	68	173	850 450 450	9 28 98	57 13 28	85 94	72 66	7 7
16	23	65	172	850 450 450	20 28 98	86 12 21	110 92	98 71	10 7

tend to be a little higher, with a range of 14 to 28 micrograms and an average value of 21 micrograms. The extra excretions after the test dose are well below those obtained with subjects with an adequate intake of thiamine. Three of the values (Subjects 9, 10 and 13) for the extra excretion following the test dose obtained after twenty-eight days are within the range of values (1 to 6 per cent of the dose) obtained with the subjects who had been given only 150 micrograms of thiamine daily for three to five months (Table I). Four values (Subjects 5, 6, 8 and 14) were outside this range by only 1 or 2 per cent and

four were in the range of 10 to 12 per cent. There were no very significant changes in the responses to the test dose after an additional period of seventy days on the same restricted intake of thiamine with the exception of Subjects 9 and 12. These two subjects returned less than 1 per cent of the test dose after ninety-eight days. At the end of the ninety-eight-day period, symptoms of thiamine deficiency were observed in all cases.

Six of the eleven subjects included in Table II were selected for study of the thiamine requirement of normal persons. However, one of the

subjects became unable to cooperate satisfactorily as a result of the previous restrictions of thiamine and, therefore, only five subjects are considered here. Two of them (5 and 6) had been given 7.5 mgm. of thiamine chloride from December 4, 1940 to January 9, 1941 (thirty-seven days). These two subjects had had opportunity to store maximal amounts of thiamine and were free from any signs of deficiency. The other three subjects had been maintained for 169 days on an intake of less than 450 micrograms daily, and their stores were undoubtedly depleted. On January 10, 1941, and thereafter until June 1, 1941, the intakes of thiamine of all five subjects were identical. The basic diet was continued and increasing amounts of thiamine chloride were given. A given level of thiamine intake was maintained until the excretions became relatively constant. The average of the values for the daily excretions at the various levels of intake is given in Table III; also the results of the test dose procedures are given in terms of percentage of the dose. The group averages of the daily excretions are presented graphically in Figure 1. It will be observed that the excretions by Subjects 5 and 6 fell to approximately the same level as the excretions of the other three subjects at the intake level of 800 micrograms and then all excretions increased at about the same rate as the intake increased. The response of Subjects 5 and 6 (Table III) to the test dose was essentially constant until the intake reached 800 to 1000 micrograms, after which the proportion of the test dose recovered in the urine

rose to an average of 39 per cent of the dose when the intake was 2 mgm. The response of Subjects 12, 13 and 16 followed a similar pattern after the intake level reached 800 micrograms. At the lower levels of thiamine intake the recovery of the test dose was much less than in the case of Subjects 5 and 6. There are unexplained fluctuations, possibly the result of technical errors, in the response to the test dose. For example, the responses of Subjects 13 and 16 show a sudden fall when the level of intake is 1000 micrograms, but this loss is more than made up in the determination at the next level of intake.

The sudden jump in the amount of thiamine excreted when the intake was increased from 800 to 1000 micrograms is worthy of note. The previous increment of 200 micrograms (from 600 to 800) produced an average increase of 45 micrograms in the excretion by Subjects 12, 13 and 16, while the excretions by Subjects 5 and 6 diminished. The next increment (from 800 to 1000), however, reversed the trend of Subjects 5 and 6 and produced an increase of 105 micrograms in the average excretion of all subjects. This finding suggests that a level of intake of 800 micrograms was just enough for physiologic purposes and that above this level a greater amount was excreted as surplus. Excretion of an average of 39 per cent of the amount ingested when the intake was 2000 micrograms indicates a considerable waste of thiamine at this level.

Since the results of Table III were obtained when all of the ingested thiamine over 400 micro-

TABLE III
Excretion of thiamine during maintenance at different levels of thiamine intake

Daily intake of thiamine	Good stores				Poor stores					
	Subject 5		Subject 6		Subject 12		Subject 13		Subject 16	
	Average daily excretion	Recovery of test dose	Average daily excretion	Recovery of test dose	Average daily excretion	Recovery of test dose	Average daily excretion	Recovery of test dose	Average daily excretion	Recovery of test dose
	micrograms	per cent	micrograms	per cent	micrograms	per cent	micrograms	per cent	micrograms	per cent
400	434	17.6	386		33	14.2	53	7.6	47	11.0
600	196	20.0	233	20.3	71	17.2	57	17.4	52	13.7
800	152	21.2	126	19.7	128	24.6	90	21.3	101	18.9
1000	241	18.6	216	26.6	133	25.0	243	12.9	190	12.4
1200	282	28.6	239	27.7	264	10.8	219	25.8	202	24.7
1400	424	30.9	309	25.4	403	21.7	335	25.3	287	17.2
1600	669	36.5	498	35.7	581	42.6	498	25.8	557	31.9
1800	699	42.0	547	44.9	591	24.4	596	36.5	590	39.0
2000	858	32.3	786	46.1	770	52.1	740	19.1	731	45.8

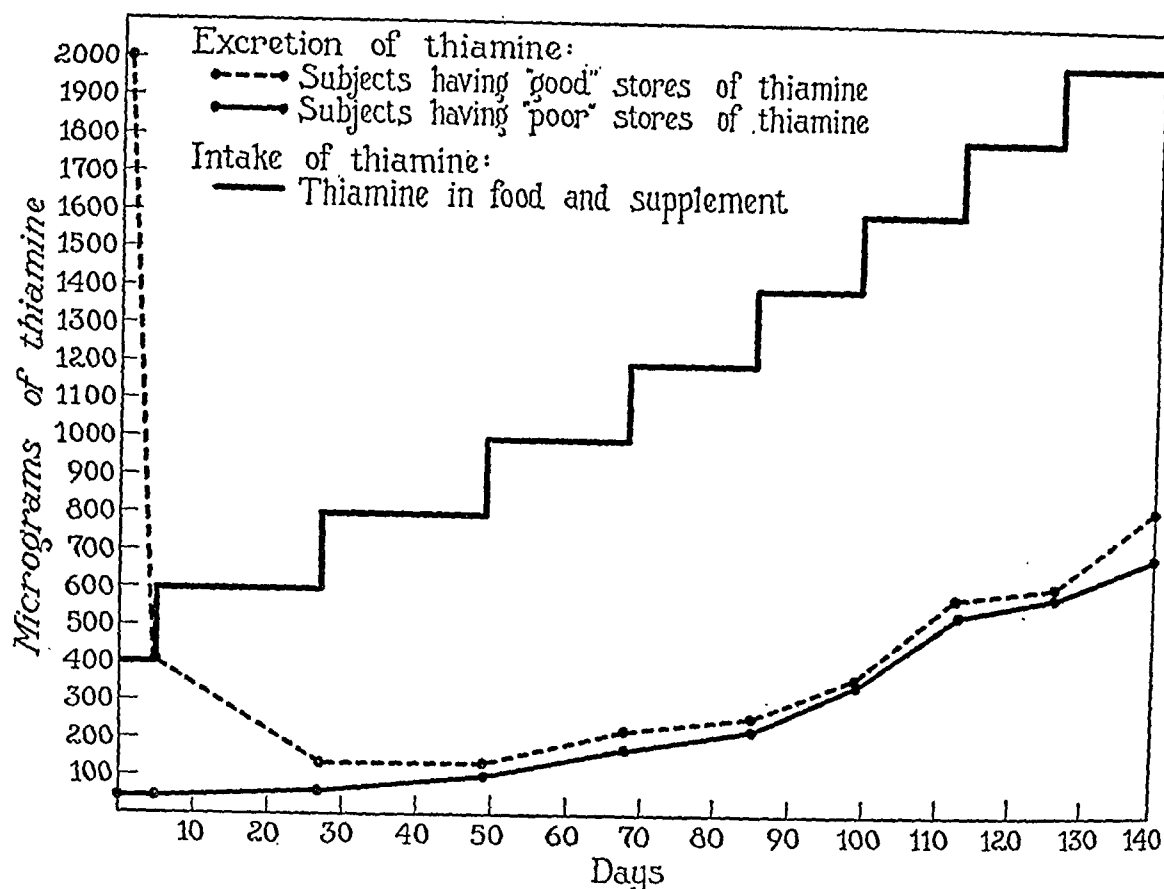


FIG. 1. EXCRETION OF THIAMINE IN THE URINE DURING STUDY OF REQUIREMENT OF THIAMINE

grams was in the form of a solution of thiamine chloride, it was necessary to determine the excretions when all of the ingested thiamine was derived from the food. When diets, which contained 1000, 1500 and 2000 micrograms of thiamine in the component foods, were consumed, the values for the urinary excretions of thiamine were within the range of values recorded in Table III at these same levels of intake. It thus appears that thiamine is utilized as effectively when it is ingested as an aqueous solution of thiamine chloride as when it is ingested as natural vitamin B₁ in foodstuffs.

There are many occasions when complete collection of urine for twenty-four hours is inconvenient or even impossible, whereas collection for a period of four hours should be readily obtained. A further study of the four-hour test is under way to determine the relation between the nutritional state with respect to thiamine and the magnitude of the response to the test dose with a four-hour collection of urine.

COMMENT

Examination of the data presented in Table I reveals that after 169 and eighty-eight days of the

severe restriction of thiamine, at which times a state of vitamin B₁ deficiency was well developed in all cases, the extra excretion of thiamine after the test dose was 1 to 6 per cent of the dose. It also should be noted that at this time the ordinary twenty-four-hour excretions were within the range of 7 to 26 micrograms. It will be observed that when the nutrition with respect to thiamine was adequate the response to the test dose with one exception (Subject 3) resulted in values for the extra excretion of thiamine of more than 20 per cent of the dose. There is thus a distinct separation of the response of the subjects in a state of marked thiamine deficiency from the response of those receiving an adequate supply of thiamine. However, the greatest value of a laboratory test as an aid to diagnosis would lie in its application to those borderline cases in which frank symptoms of a state of deficiency are not evident.

The value of measurement of the excretion of thiamine and of the test-dose procedure may be estimated by consideration of the clinical condition of the subjects in Table II in conjunction with the laboratory data. Fatigue, lassitude, anorexia and mental depression were evident in some instances

at the twenty-eighth day but a definite diagnosis of thiamine deficiency could not be made at that time on the basis of these symptoms or other laboratory data. The urinary excretions furnished the only unequivocal evidence of thiamine deficiency. On the ninety-eighth day the signs and symptoms and other laboratory data which have been described elsewhere were sufficiently definite to merit a diagnosis of thiamine deficiency without reference to the data on the urinary excretion. When a state of thiamine deficiency has progressed to such a degree that it can be diagnosed from symptoms alone, determination of the urinary excretion has merit as a confirmatory procedure, but in the differentiation between a state of thiamine deficiency similar to that on the twenty-eighth day and other conditions which give rise to like symptoms, determination of the urinary excretion can be the decisive factor.

It is evident from the results presented that, in general, the ordinary excretion affords as much information as to the physiologic state with respect to thiamine as does the test-dose procedure. It is of value, however, to have the results of both determinations when possible and, of course, it is necessary to determine the ordinary excretion prior to administration of the test dose when twenty-four-hour collections of urine are made.

It is to be noted that the usual consequence of a restriction of the dietary intake of thiamine to 450 micrograms or less is a rapid fall in the values for the ordinary excretion of thiamine during twenty-four hours and in the recovery of the test dose in the urine. Since there is this close correlation between immediate intake and excretion both before and after a test dose, an estimate of the duration of the deficient intake of thiamine cannot be made on the basis of excretion studies.

We have previously reported that the excretions by Subjects 1, 2, 3 and 4 remained at essentially a normal level for approximately three months after restriction of the thiamine intake. The amount of thiamine excreted by all other subjects rapidly decreased when the ordinary diet was changed to one which furnished an inadequate amount of thiamine. We are unable to explain the divergence of our first observations from our later ones which are in agreement with the observations of other investigators (15, 16, 17).

The foregoing statements do not necessarily apply, however, when the intake of thiamine has been much greater than the minimal requirement for a month or more. Subjects 5 and 6 (Table III) furnish examples of what the excretions may be under these circumstances. Subjects 5 and 6 received 7.5 mgm. of thiamine chloride for thirty-seven days. Then they were placed for five days on a diet which furnished 400 micrograms of thiamine and for twenty-two days on a diet which furnished 600 micrograms. The intake of thiamine was thus definitely deficient, as judged by all the criteria available, for twenty-seven days, yet at the end of that period the twenty-four-hour excretions were 140 and 107 micrograms, respectively, and 20 per cent of the test dose was recovered in the urine. These values ordinarily would be considered indicative of an adequate intake. When these values are compared with the corresponding values of 88, 38 and 65 micrograms for Subjects 12, 13 and 16 and with recoveries of 14 to 17 per cent of the test dose, it is obvious that Subjects 5 and 6 must have been drawing upon a store of thiamine. In these instances the excretions and responses to the test dose did not indicate the inadequate intake but did show adequate nutrition with respect to thiamine of these two subjects.

The results obtained with Subjects 5, 6, 8 and 9 (Table I), after treatment with more than 10 mgm. of thiamine daily for thirty-three days, also give evidence of a retention of thiamine which resulted in elevated twenty-four hour excretions after five days on a diet which furnished 850 micrograms of thiamine and which also resulted in elevated, although substandard, responses to the test-dose procedure after a further period of five days with an intake of 150 micrograms. The decrease in the response to the test-dose procedure during the latter period again illustrates the usual close relation between the level of intake of thiamine and this response.

As previously mentioned, the data in Table III suggest that the *minimal* intake of thiamine to supply the physiologic needs of five of these subjects was 800 micrograms, or 0.4 mgm. per thousand calories. At this level, the average value for the twenty-four-hour excretion was 119 micrograms and the average recovery of the test dose was 21 per cent. Allowing for inaccuracies in the

OSMOMETRIC BEHAVIOR OF NORMAL HUMAN ERYTHROCYTES

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METHOD

It is generally accepted that when erythrocytes are suspended in hypotonic solutions they first take up water and swell, and then at some critical tonicity burst and allow hemoglobin to escape. While there are many indications that the permeability of these cells to both salts and water may vary considerably in different conditions (1, 2, 3), quantitative data on the osmometric behavior of either normal or abnormal erythrocytes are scarce. Methods used in clinical studies of the osmotic fragility of erythrocytes are mostly limited to qualitative observations of hemolysis as an indication of the rupture of the cells.

Quantitative methods for estimating the amounts of hemoglobin liberated from erythrocytes in salt solutions of different tonicities were devised in 1903 by Arrhenius and Madsen (4) and in 1921 by Hastings (5), each making use of visual colorimetry; and recently Waugh and Asherman (6) and Hunter (7) used photoelectric colorimetry for the same purpose. Such methods permit the estimation of the number of cells ruptured at each step of a hemolytic series but tell nothing of the osmotic behavior of the cells during stages of their swelling up to the point of rupture. In 1939 we developed a method (8) for measurement of changes in volume of erythrocytes in hypotonic salt solutions, but this method did not account for the swelling of cells remaining intact after hemolysis of part of the cells at critical tonicities. That method has since been elaborated, as here described, to include quantitative measurements* of hemolysis as well as the volume of the red cells at each tonicity in a series. These parallel determinations permit the calculation of the swelling of intact cells both before hemolysis begins and in solutions where partial hemolysis has occurred. Data obtained by this method in studies of normal human erythrocytes are reported here. Data on abnormal types of human erythrocytes and erythrocytes of a number of other species will be reported later.

Apparatus. The modified Van Allen hematocrit tube illustrated in Figure 1 contains 8.0 cc. when filled to the upper mark, and the graduated portion of the stem (100 divisions, numbered by tens) contains 0.02 cc. It is necessary that the bulb of the pipette should slope steeply and smoothly into the stem so that no cells will stick at the shoulder during centrifugation. For centrifugation of a series of the pipettes, we use the International Centrifuge Type SB, No. 2, with 8-place head and 3-place trunnion carriers. The pipettes, each sealed with a spring clip as shown in Figure 1, are placed in 15 cc. centrifuge tube holders. The Evelyn photoelectric colorimeter (9) is used with the light filter 540 for measurement of hemoglobin concentrations.

Solutions. Salt solutions of different concentrations varying from 0.9 to 0.1 per cent sodium chloride are prepared and kept as described previously (8).

Blood samples. Either defibrinated or heparinized blood may be used. As stated in the earlier paper (8), best results are obtained if the volume of cells is above 30 per cent.

Procedure. Fit a rubber suction tube with mouthpiece to the upper end of the pipette and draw blood into the stem to the 100 mark. After wiping excess blood from the tip draw the blood into the bulb and, after it, the appropriate salt solution to fill the bulb almost to the 8.0 cc. mark. By not filling the pipette quite to the mark at this stage, an air bubble is left in the bulb to facilitate mixing. Mix by inverting the pipette repeatedly, with a finger placed over the upper end. Make the dilution of blood in 0.9 per cent NaCl solution in duplicate. Fill thus an appropriate number of pipettes (usually a series of 12) with solutions of NaCl decreasing in concentration to that which gives complete hemolysis as judged by the eye. Choice of decrements in concentrations may be made to suit the needs of individual experiments, the smaller decrements of 0.025 per cent being used in the range where hemolysis is expected to occur. In the same manner, dilute the blood with distilled water in the last pipette of the series. Keep the filled pipettes in a horizontal position for one hour. Since the cells settle and may adhere to the glass, resuspend them at the end of the hour by repeatedly inverting the pipettes as before.

Centrifuge the pipettes 20 minutes at 2500 to 3000 r.p.m., and read the height of the column of packed red cells in per cent (see Table I). Add distilled water through the top of each pipette to bring the fluid to the 8.0 cc. mark. Then withdraw the fluid from each bulb by means of a long thin-stemmed pipette and transfer it to a colorimeter tube. To insure absence of turbidity, add

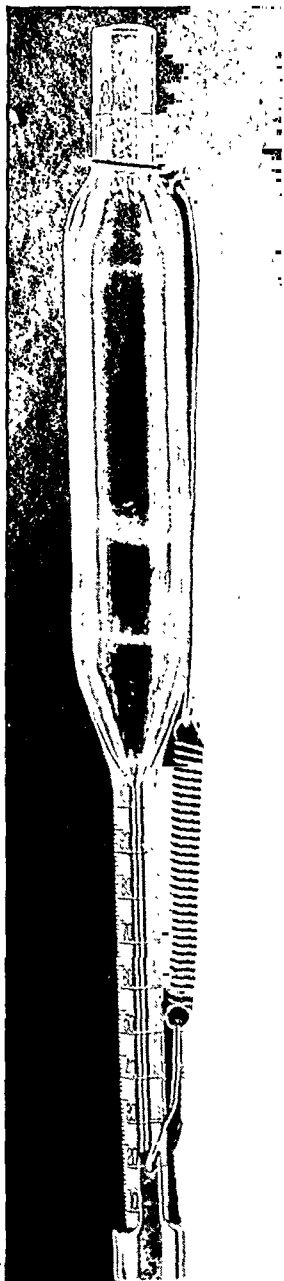


FIG. 1. BLOOD DILUTING PIPETTE, A MODIFIED VAN ALLEN HEMATOCRIT TUBE (14)

Made by Macalaster Bicknell Company, 243 Broadway, Cambridge, Mass.

a drop of concentrated ammonium hydroxide to each, a few minutes before the colorimetric readings are to be made. Read the light transmissions in the Evelyn instrument, with the filter 540. Use the fluid from the first pipette of the series (0.9 per cent NaCl) as the blank for adjustment of the galvanometer. Light transmission of this fluid is usually slightly less than that of distilled water, presumably owing to substances in the blood plasma.

To determine the total hemoglobin content of the blood dilute a portion 1:400 in distilled water, and transfer this "total" hemolysate, uncentrifuged, to a colorimeter tube.

Shortly before reading its limit transmission, add to it a drop of concentrated ammonium hydroxide to insure both complete hemolysis and absence of turbidity.

For calculating the per cent hemolysis in each fluid of the series, all the galvanometer readings may be converted to hemoglobin values by means of a previously established factor or calibration curve, and these in turn converted to percentages of the total hemoglobin; or more directly, the density or L value ($L = 2 - \log G$) of each fluid may be divided by that of the total hemolysate to obtain the per cent hemolysis.¹

Divide the readings of the height of the column of cells in the stem of each pipette by that of the first tube, and multiply by 100 to express the cell volumes in percentages of the initial volume of the cells in 0.9 per cent NaCl solution.² Where partial hemolysis has occurred, calculate the volume of the intact cells by correcting the observed volumes for the loss of cells by hemolysis. Table I illustrates the tabulation of data thus obtained on the erythrocytes of a normal infant. For example, in 0.425 per cent NaCl solution the cells in the stem of the pipette occupied a volume (reading 55.6) equal to 142 per cent of the volume of cells in the first pipette; but these intact cells were only 81.6 per cent ($100 - 18.4$ per cent hemolysis) of the original number. Therefore, $142 \div 81.6 \times 100 = 174 =$ the corrected volume of the intact cells in per cent of their initial volume.

¹ In some instances the advisability of using the hemoglobin content of the centrifuged water hemolysate (the last tube of the series), as representing complete hemolysis, might be considered. With normal bloods the difference between this and the total hemoglobin is small, but the difference varies greatly with different types of blood, and is greatest with the erythrocytes of sickle cell anemia; in some cases the centrifuged water hemolysate may contain only 90 per cent or less of the total hemoglobin. Ponder has called the hemoglobin thus centrifuged out "residual hemoglobin" and finds that it is adsorbed on the ghosts (personal communication).

² For the studies described later under RESULTS, the volume of cells in each sample of heparinized blood was determined by centrifugation of the undiluted blood in capillary tubes at about 12,000 r.p.m. for 5 minutes (10). The cell volume thus determined was usually from 0.5 to 2 volumes per 100 volumes of blood lower than the volume read in the first Van Allen tube; such a difference might be due to a difference in packing of the cells at different speeds (although longer spinning in the Van Allen tubes did not pack the cells further) or to a slightly higher real volume of the cells in the 0.9 per cent NaCl solution. As a basis for comparison of different bloods, it was deemed preferable to calculate the volumes of the cells in the series of hypotonic solutions in percentages of their volume in 0.9 per cent NaCl solution (the first tube) rather than in the undiluted blood. For some purposes, however, it may be better to use the cell volume determined in the undiluted blood as the initial value.

TABLE I

Hemolysis and changes in volume of normal erythrocytes diluted 1:400 with solutions of sodium chloride

Initial mean dimensions of these cells are listed as the first example of normal infant's blood in Table II, and the changes are shown graphically, for comparison with expected swelling of the cells, in Figure 2.

NaCl	Hemo-lysis	Volume of cells		
		Read-ings	Uncorrected for hemolysis	Corrected for hemolysis
per cent	per cent	per cent	per cent of volume in first tube	per cent of volume in first tube
0.900	0	39.2	100	
0.800	0	42.5	108	
0.700	0	47.2	120	
0.600	0	51.4	131	
0.550	0	55.4	141	
0.525	0	58.6	149	
0.500	0.6	60.4	154	155
0.475	4.0	62.4	159	166
0.450	9.3	61.0	156	172
0.425	18.4	55.6	142	174
0.400	45.8	35.0	89	165
0.375	65.6	19.4	50	144
0.350	80.5	7.6?	19?	99?
0.325	91.5	?	0?	
H ₂ O	97.9			

APPLICATIONS OF THE METHOD AND RESULTS

In applying the method to studies of the osmometric behavior of erythrocytes, further measurements and calculations were made following a plan of study suggested in recent papers by Haden (11, 12) and Ponder (2, 3).

The red cells in each sample of heparinized blood were counted with a hemocytometer as usual, their volume in the undiluted blood was determined by the method of Guest and Siler (10), and their mean volume was calculated in the usual manner by dividing the volume of the cells by their number per c.mm. of blood. The mean diameter of the cells was read from stained films by means of the Haden-Hauser erythrocytometer (13) with a drop of 0.9 per cent NaCl solution and cover glass placed over the blood film. From values for the mean volume and mean diameter, the mean thickness of the cells was calculated by the formula $T = V/\pi r^2$. Their mean surface area was calculated as that of a flat disc of the given thickness and diameter, and the volume of a sphere with this surface area was calculated. The per cent swelling which the cells would undergo to become spheres with the same mean surface area was then obtained from the ratio of their initial mean volume to that of the spheres to which they

might be converted without exceeding this surface area (*i.e.*, without rupture of the cell membranes). The latter value was designated "expected maximal volume."

The "expected osmometric volume" of the cells at each tonicity (*i.e.*, the volume they should attain if they behaved as perfect osmometers) was calculated according to Ponder's formula: $V = W(1/T - 1) + 100$; where V = the new volume of the cells in per cent of their initial volume; W = the percentage by volume of water in the cells, taken as 70 per cent; T = the ratio of the tonicity of the medium to 0.9 per cent NaCl solution (*i.e.*, with 0.45 per cent NaCl, $T = 0.5$); 100 = the initial volume of the cells.

Figure 2 illustrates the swelling and hemolysis of the erythrocytes of a normal infant (data in Table I). The swelling (curves 2 and 3) closely

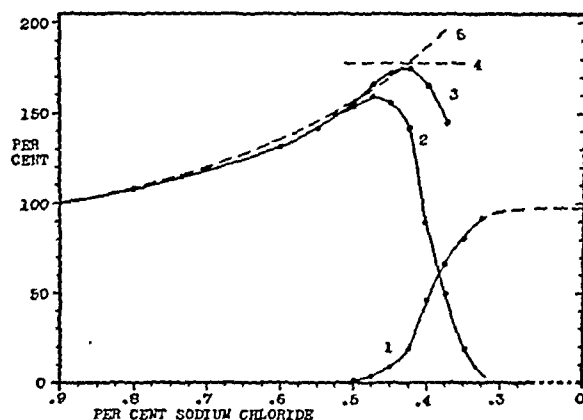


FIG. 2. THE BLOOD OF A NORMAL INFANT, J. P., 14 MONTHS OF AGE; HEMOLYSIS AND CHANGES IN VOLUME OF ERYTHROCYTES IN HYPOTONIC SALT SOLUTIONS (DATA LISTED IN TABLE I)

Hemolysis is expressed in per cent of the total hemoglobin in the sample. Cell volumes are expressed in per cent of the initial volume of the cells in 0.9 per cent NaCl solution. The symbols represent, respectively: 1. Hemolysis; 2. Observed cell volume; 3. Cell volumes corrected for hemolysis, *i.e.*, the volume of the unhemolyzed cells; 4. Expected maximal mean cell volume calculated from the mean surface area according to Haden; 5. Expected osmometric volume of the cells at different tonicities, calculated according to Ponder, assuming the initial water content of the cells to be 70 per cent.

³ In different papers on this subject Ponder used the figures 67 per cent (2) and 60 per cent (3) for the water content of rabbit erythrocytes. Our own data on the water content of human erythrocytes, determined by drying at 105°, average around 70 per cent.

TABLE II

The swelling, expected and found, of normal human erythrocytes in hypotonic salt solutions

Subjects	Initial mean dimensions				Volume of a sphere with same surface area	Tonicity at which maximal swelling occurred	Expected osmometric volume at this tonicity ▲	Maximum cell volume	
	Volume	Diameter	Thickness	Surface area				Expected, calculated from surface area	Found, observed volume corrected for hemolysis
	μ^3	μ	μ	μ^2	μ^3	per cent NaCl	per cent	*per cent	*per cent
Normal adults	90	7.7	1.93	140	156	0.425	179	173	179
	92	7.7	1.98	141	157	0.450	170	171	172
	88	7.7	1.89	139	154	0.425	179	175	175
	86	7.6	1.94	137	151	0.450	170	176	178
	91	7.7	1.95	140	156	0.425	179	172	180
	87	7.6	1.92	137	151	0.425	179	174	172
	94	7.8	1.97	144	162	0.425	179	172	175
Normal infants and children	82	7.6	1.81	134	146	0.425	179	178	174
	77	7.4	1.79	128	136	0.425	179	177	174
	77	7.4	1.79	128	136	0.425	179	177	172
	84	7.5	1.90	133	144	0.425	179	174	173
	82	7.5	1.86	132	143	0.425	179	174	173
Normal newly born infants	110	8.1	2.14	157	185	0.450	170	168	165
	108	7.7	2.32	149	171	0.450	170	158	155
	109	7.7	2.34	150	173	0.475	162	159	153

▲ Volume of a perfect osmometer, in per cent of its initial volume, assuming the initial water content to be 70 per cent by volume.

* Values in per cent of the initial volume of the cells determined in 0.9 per cent NaCl solution.

approached at all points that expected in a perfect osmometer (curve 5), and the maximum swelling at the peak closely approached that predicted from the surface area (broken line 4).

In Table II are listed data on several samples of erythrocytes of normal adults, children, and infants. They are arranged for comparison of the maximal volumes found in each case with the respective "osmometric" volumes expected at the tonicity where maximal swelling was found, and the expected maximal volumes calculated from the initial mean dimensions cited. The maximal volumes attained by the erythrocytes of normal subjects were about 175 per cent of the respective initial volumes, and among these there was little variation from this figure. In most instances the maximal swelling was attained in 0.425 per cent NaCl solution, and it was rarely found outside the range from 0.475 to 0.400 per cent NaCl. The few observations made on the erythrocytes of newly born infants (large cells) indicated that the osmotic behavior of these cells was approximately like that of the normal erythrocytes of older individuals, although the maximal swelling of these

cells was somewhat less, per centum, than that of the smaller erythrocytes of adults.

Effects of varying dilutions and of washing the cells. The maximal swelling and beginning hemolysis of erythrocytes diluted with hypotonic salt solutions in the 8.0 cc. pipettes were found to occur at tonicities of salt solution slightly higher (about 0.025 per cent of NaCl) than in the original Van Allen pipettes with smaller bulbs, used as described in our earlier paper (8). The dilution of blood is approximately 1:100 in the original type of pipette, and 1:400 in the 8.0 cc. pipette. The maximum swelling of the cells was, however, the same at both dilutions.

When normal erythrocytes were washed by centrifugation in 0.9 per cent NaCl solution, resuspended in the salt solution (to give about 30 per cent volume of cells), and then tested as here described, the washed cells tended both to swell and to be hemolyzed at a slightly higher tonicity than the unwashed cells; but the maximum volume attained by the washed and unwashed cells was the same. Further studies of the effects of washing normal and abnormal types of erythrocytes

with salt solutions of different tonicities have been made and will be reported later.

DISCUSSION

The foregoing observations on normal human erythrocytes support the general validity of two principles which have been formulated regarding the swelling of these cells in hypotonic salt solutions. The thesis of Ponder and others, that erythrocytes behave within certain limits as perfect osmometers, is supported by the close agreement found between values for "expected osmometric swelling" and for the cell volumes determined at each tonicity. The thesis of Haden and others that the maximum swelling of erythrocytes is limited by an inelastic surface membrane is supported by the close agreement found between values for the maximum swelling expected, *i.e.*, calculated from the mean surface area of the cells, and the maximum cell volumes determined in each of the examples cited here. Similar studies of other types of erythrocytes have, however, yielded results indicating that the osmotic behavior of certain abnormal red cells does not conform to these principles. Data on abnormal human erythrocytes will be reported in a later paper.

The shapes of the curves 2 and 3 in Figure 2, representing changes in volume of the cells after the point of beginning hemolysis, lead to speculation on the way in which cells of any given sample may differ amongst themselves in their osmotic behavior. Since the observed swelling, up to the point of beginning hemolysis, corresponds closely to that expected in a perfect osmometer, it would appear that all the cells in normal bloods swell proportionately alike in all solutions of the series up to this point. There exists a possibility, however, that the observed swelling merely represents the mean, and that at different tonicities some cells swell more and some less than the average cells in the whole sample. The fact that the curve 3 falls off in the stages following about 50 per cent hemolysis suggests that the cells remaining unhemolyzed at this point either did not swell to the same degree as the others or that they became smaller after attaining a maximal volume.

Several explanations may be offered to account for the apparent dissimilarities in osmotic be-

havior thus observed among the cells of one sample. Due to different physical or chemical characteristics, the more resistant cells (*i.e.*, those hemolyzed at lower tonicities) might not follow the osmotic law and their swelling might therefore lag behind that of the cells which hemolyze first. The dimensions of the unhemolyzed cells might not correspond to the recorded mean measurements, in which case their expected maximal swelling would differ from that predicted from the mean measurements of all cells in the sample. There is a possible fallacy in the assumption that 10 per cent hemolysis, measured by the amount of hemoglobin in the supernatant fluid, represents the rupture of 10 per cent of the initial number of cells in the sample; this amount of hemoglobin would not represent the same proportion of cells if it were derived all from small cells or all from big cells. While it is generally believed that the hemolysis of individual erythrocytes is "all or none" at the point of rupture, it is conceivable that the more resistant cells may lose part of their contents without the rupture needed to liberate hemoglobin; thus the salts might escape from some cells more easily than from others, leading to less swelling if this occurred before the peak, or to shrinkage if it occurred when they were swollen to the maximum. Such considerations assume greater importance with regard to erythrocytes found in diseases characterized by abnormal red cell fragility.

To see whether differences in certain characteristics of the more resistant cells could be demonstrated, the following experiments were made. Samples of blood drawn from normal persons were diluted in centrifuge tubes with hypotonic salt solutions of tonicities chosen to produce from 25 to 72 per cent hemolysis. After standing an hour the tubes were centrifuged, the supernatant fluid was drawn off (its hemoglobin content was determined to measure the extent of hemolysis), and the cells were resuspended in a small volume of physiological salt solution. Determinations of cell count, cell volume, and hemoglobin content of this preparation were made and values calculated therefrom for the mean volume, mean hemoglobin concentration, and mean hemoglobin content of the unhemolyzed (more resistant) cells. These values did not differ notably from the mean values

determined on all the cells of the original samples. While it thus appeared that the resistant cells did not differ materially in these characteristics from the less resistant ones, subsequent tests of these cells showed a slightly different behavior with regard to swelling and hemolysis when resuspended in the series of hypotonic solutions. Further studies of the effects of washing cells with hypotonic saline will be reported in later communications.

SUMMARY

Parallel measurements of the swelling and hemolysis of erythrocytes suspended in hypotonic salt solutions were made by means of a modified Van Allen hematocrit tube with a bulb calibrated to contain 8.0 cc. of fluid.

The swelling of normal human erythrocytes thus measured followed closely that expected if they behave as perfect osmometers.

The maximum volumes attained by normal human erythrocytes agreed closely with predictions based on their mean surface area calculated from their initial mean volume, diameter, and thickness, thus supporting the view that the erythrocyte cannot distend beyond the limit set by its surface area.

BIBLIOGRAPHY

1. Davson, H., Loss of potassium from the erythrocyte in hypotonic saline. *J. Cell. and Comp. Physiol.*, 1937, 10, 247.
2. Ponder, E., Mammalian red cell and the properties of haemolytic systems. *Protoplasma-Monographien*, 1934, 6, 117.
3. Ponder, E., Red cell as an osmometer. *Cold Spring Harbor Symp., Quant. Biol.*, 1939, 8, 133.
4. Arrhenius, S., and Madsen, T., Anwendung der physikalischen Chemie auf das Studium der Toxine und Antitoxine. *Ztschr. f. physik. Chemie*, 1903, 44, 7. (Quoted by Harvey, W. F., Studies in method and standardization of blood examination; estimation of erythrocyte fragility and normal standard. *Edinburgh M. J.*, 1937, 44, 100.)
5. Hastings, A. B., Physiology of fatigue; physico-chemical manifestations of fatigue in the blood. *U. S. Public Health Bull. No. 117*, Government Printing Office, Washington, 1921.
6. Waugh, T. R., and Asherman, E. G., Use of index of hemolysis in expressing fragility of erythrocytes. *J. Lab. and Clin. Med.*, 1938, 23, 746.
7. Hunter, F. T., Photoelectric method for quantitative determination of erythrocyte fragility. *J. Clin. Invest.*, 1940, 19, 691.
8. Guest, G. M., and Wing, M., Method for determination of erythrocyte fragility, using Van Allen hematocrit tubes for measurement of changes in volume of cells in hypotonic salt solutions. *J. Lab. and Clin. Med.*, 1939, 24, 850.
9. Evelyn, K. A., Stabilized photoelectric colorimeter with light filters. *J. Biol. Chem.*, 1936, 115, 63.
10. Guest, G. M., and Siler, V. E., Centrifuge method for determination of volume of cells in blood. *J. Lab. and Clin. Med.*, 1934, 19, 757.
11. Haden, R. L., Nature of hemolytic anemia. *Univ. Wisconsin, Symposium on Blood*, 1939, p. 83.
12. Haden, R. L., Studies in Hematology. University of Kansas, Extension Division, 1940.
13. Haden, R. L., New instrument for diffractometric measurement of diameter of red blood cells. *J. Lab. and Clin. Med.*, 1940, 25, 399.
14. Van Allen, C. M., Hematocrit method. *J. Lab. and Clin. Med.*, 1925, 10, 1027.

THE EXCRETION OF SPECIFIC FLUORESCENT SUBSTANCES IN THE URINE IN EXPERIMENTAL NICOTINIC ACID DEFICIENCY ^{1,2}

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In a recent publication (1) the authors have described the presence in urine of certain fluorescent substances which were found to vary in a characteristic way in pellagra. One of these substances, designated F_1 , was found in relatively small amounts in normal urine but in larger quantities in pellagra. The second substance, designated F_2 , developed fluorescence only on the addition of alkali. F_2 was not found in the urine of pellagrins but reappeared after nicotinic acid therapy. The administration of nicotinic acid to normal subjects caused an increased elimination of F_2 . These facts are summarized in Table I, which also gives the characteristics of the fluorescent spectra of the two substances with ultraviolet light. Photographs of the fluorescent spectra are shown in Figure 1.

To explain these phenomena it was suggested that F_1 was converted into F_2 through the agency of some nicotinic acid containing enzyme, a deficit of nicotinic acid causing the accumulation of F_1 which could no longer be converted into F_2 . It was further suggested that the accumulation of

F_1 might be related to the photosensitivity of the skin seen in pellagra.

Since natural pellagra is seldom an uncomplicated nicotinic acid deficiency (2), it seemed desirable to study the excretion of these fluorescent substances in animals with a pure nicotinic acid deficiency.

EXPERIMENTAL

Four dogs were employed in this study. These animals had previously been used for a nicotinic acid deficiency experiment, but had subsequently received treatment with nicotinic acid. There was no reason to believe that their recovery was not complete at the onset of the present study. Throughout the course of the study the animals received unlimited quantities of a black-tongue diet consisting of:

	per cent
Yellow corn meal	65
Cow peas	8
Purified casein	7.5
Sucrose	7
Cotton seed oil	5
Cod liver oil	5
NaCl	1
CaCO ₃	1.5

TABLE I

Features of fluorescent substances as observed in human urine

	F_1	F_2
	Little in normal urine	Much in normal urine
	Abundant in pellagra	Absent in pellagra
Fluorescence	Whitish, violet blue	Greenish blue
Range of fluorescent emission	4000-4800 A°	4200-5400 A°
Maximum emission	4350 A°	4550 A°

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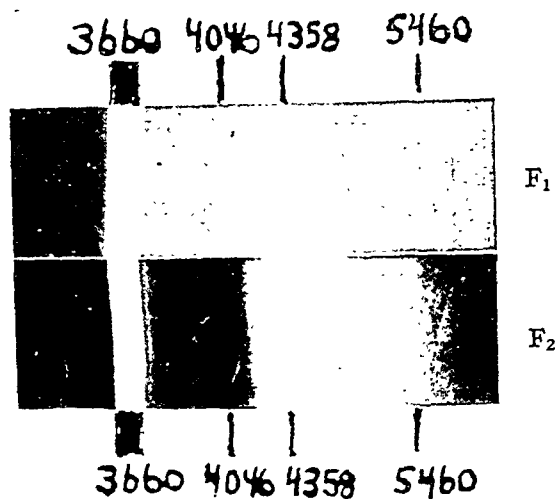


FIG. 1. PHOTOGRAPH OF THE FLUORESCENT SPECTRA

In addition, supplements of thiamin and riboflavin were given to each dog twice a week in doses of 0.175 mgm. per kilogram. The dogs were put in metabolism cages every few days in order to obtain 24-hour specimens of urine. The urine was collected in bottles containing glacial acetic acid (3 per cent of the anticipated daily volume of urine) and was assayed for F_1 and F_2 in quinine units by procedures described elsewhere (1, 3).

RESULTS

The course of the F_1 and F_2 excretion in these animals is shown in Figures 2, 3, 4 and 5. It may be noted that in all but one of the animals

the F_2 excretion at the start of the experiment exceeded that of F_1 . As the experiment continued, F_2 excretion gradually decreased to zero; this occurred more rapidly in the small dog than in the larger animals. The excretion of F_2 remained at the zero level unless nicotinic acid was given. Coincident with the fall of F_2 in the urine, there was a rise in the excretion of F_1 , which reached a maximum at or shortly after the time that the F_2 output ceased. This maximum F_1 output was, however, not sustained; as the diet continued, the F_1 showed a gradual decline to

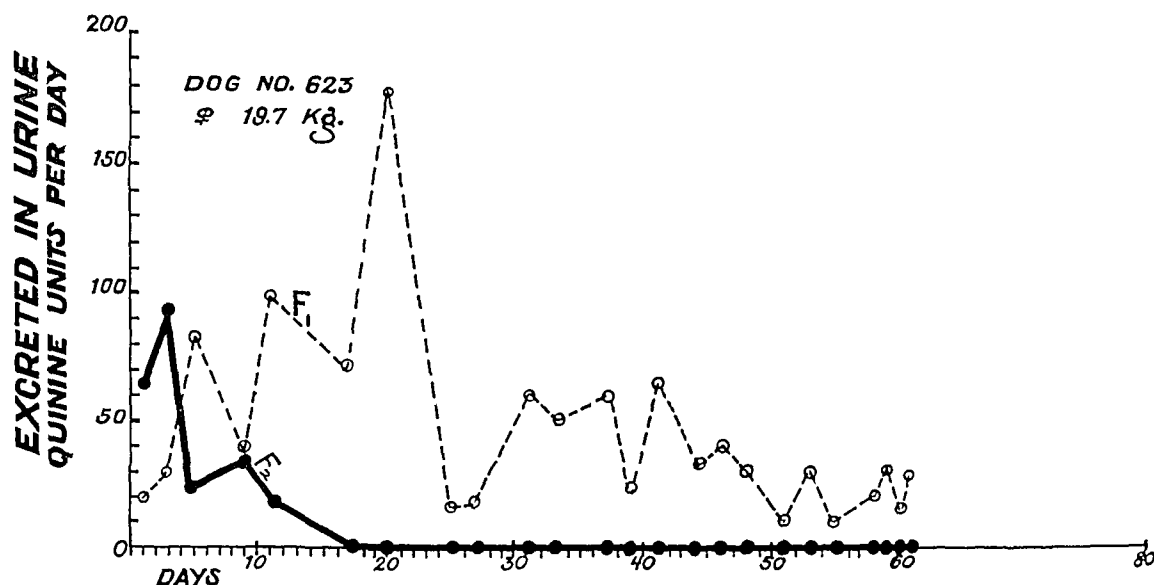


FIG. 2. EXCRETION OF FLUORESCENT SUBSTANCES IN EXPERIMENTAL NICOTINIC ACID DEFICIENCY

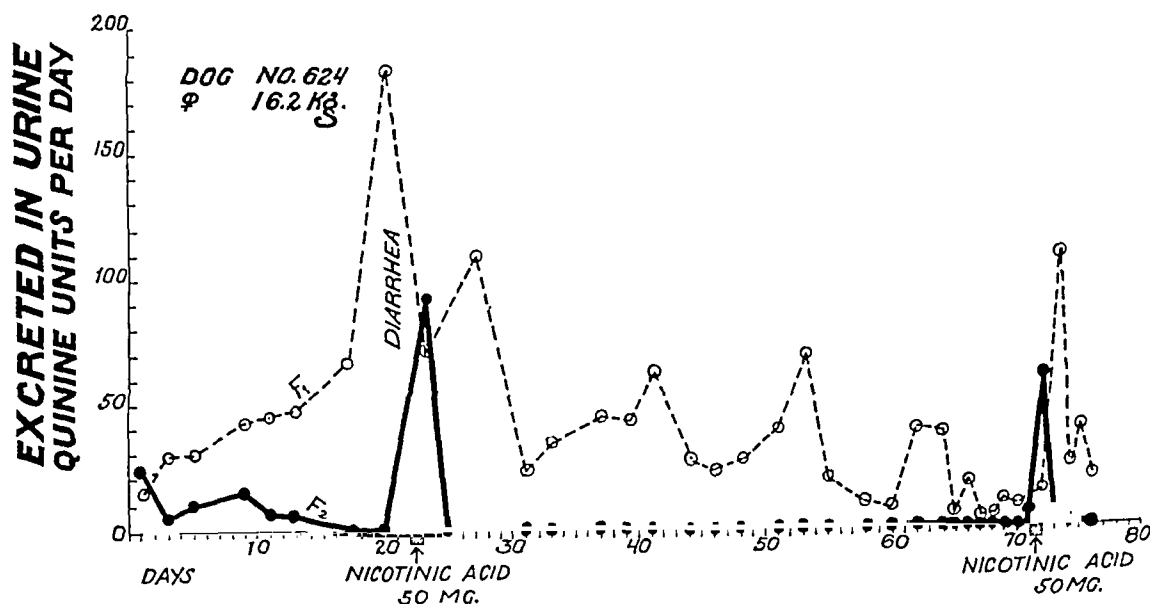


FIG. 3. EXCRETION OF FLUORESCENT SUBSTANCES IN EXPERIMENTAL NICOTINIC ACID DEFICIENCY

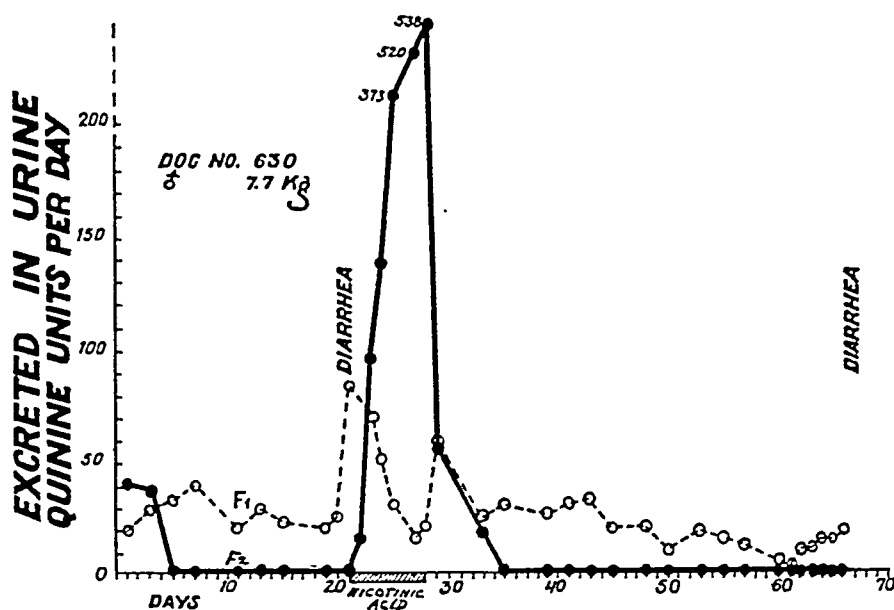


FIG. 4. EXCRETION OF FLUORESCENT SUBSTANCES IN EXPERIMENTAL NICOTINIC ACID DEFICIENCY

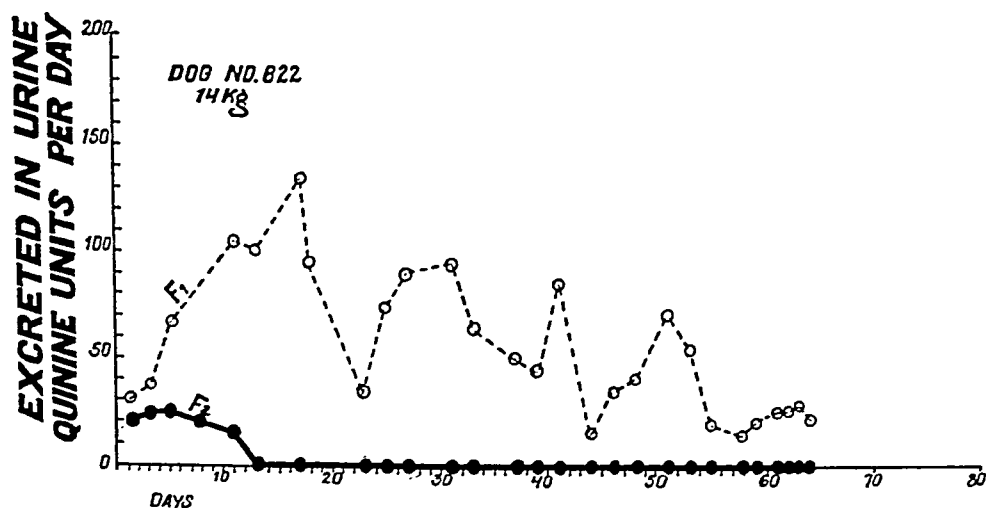


FIG. 5. EXCRETION OF FLUORESCENT SUBSTANCES IN EXPERIMENTAL NICOTINIC ACID DEFICIENCY

values approximating those at the onset of the experiment.

The effect of nicotinic acid therapy is shown in Dogs 624 and 630. The former animal developed diarrhea on the 20th day of the experiment and was subsequently given a single 50 mgm. dose of nicotinic acid orally. This medication caused a cessation of the diarrhea, a prompt rise in F_2 and a diminution in F_1 excretion. The F_2 ex-

cretion was shortlived, however. In the next specimen it had again disappeared and F_1 had increased, although the high F_1 excretion was not sustained. On the 71st day of the experiment a second 50 mgm. dose of nicotinic acid was given. This too, was followed by the prompt reappearance of F_2 in the urine. The F_1 , however, which had by then reached a low level, did not fall as a result of this dose. Indeed, it may be noted that

it showed no change at all for 24 hours, but following this exhibited a sharp but transitory increase.

The small dog Number 630 showed, in general, a similar response to nicotinic acid. On the 21st day of the experiment he developed diarrhea. His F_1 excretion had by that time risen from 20 to 170 units per day, and his F_2 had fallen to zero. He was then given 50 mgm. nicotinic acid a day by mouth for one week. This resulted in a rise of F_2 to extraordinarily high levels and in a simultaneous decrease of F_1 . Following the withdrawal of nicotinic acid, the F_2 excretion fell to zero in the course of a week, and F_1 , after a temporary rise, continued to decline steadily until the termination of the experiment.

COMMENT

The observations made on these animals confirm those made on 4 pellagrins studied by us (Table II). The dog experiments, like the studies of patients, demonstrate the absence of F_2 excretion and the tendency of F_1 to rise. Somewhat unexpected, however, was the observation made on the dogs that the rise in F_1 was not indefinitely sustained. In examining the human data from this point of view, it is worthy of note that the most severe case of all, as judged by history, symptoms and reappearance of F_2 in the urine after therapy (Patient E), did not show as high an F_1 excretion as did another definitely milder case (Patient S). It now seems likely that the stage of high F_1 excretion had been passed in this severe case, and that the condition was comparable to that observed in our dogs after many weeks on the experimental diet.

Although, in general, a reciprocal relation between F_1 and F_2 excretion seems to hold, it is now apparent that this relationship is not a perfect one. The fall in F_2 on withdrawing nicotinic acid is not always accompanied by an equally prompt and impressive rise in F_1 (Dog 630). Likewise the administration of nicotinic acid may cause a rise in F_2 excretion far greater than the fall in F_1 (Dog 630) or may under other circumstances (Patient E) cause a reduction in F_1 without any corresponding rise in F_2 .

In interpreting these facts, one must bear in mind the possibility that one or both of these substances may be of physiological importance, and that the failure of one to appear quantitatively as the other disappears may be due to a demand made by the body which results in utilization rather than excretion. It is also possible that intermediary non-fluorescent compounds are formed in the conversion of F_1 to F_2 . Furthermore, one cannot deny the possibility that the reactions causing the disappearance of F_1 and the appearance of F_2 are two independent reactions, both of which are catalyzed by nicotinic acid.

The gradual decrease in F_1 excretion in the later stages of the deficiency also demands an explanation. In the absence of knowledge of the chemical nature or precursors of this substance, this can hardly be discussed with profit at the present time. It is hoped that studies now in progress will throw some light on the subject.

SUMMARY

The fluorescent substances F_1 and F_2 have been followed in the urine of dogs with experimental

TABLE II
*Urinary excretion of F_1 and F_2 in pellagrins **
(Expressed in quinine units)

Subject	Severity of symptoms	Before treatment		After 50 mgm. nicotinic acid by mouth	
		F_1	F_2	F_1	F_2
W.	Very mild	12	0	22	18
H.	Moderately severe	38	0	26	17
S.	Moderately severe	104	0	60	11
E.	Very severe	64	0	40	0
Normal control subjects		10-15	20-35	15-20	35-50

* Figures represent excretion during a 4-hour period.

nicotinic acid deficiency. Observations on these animals are in agreement with those made in human pellagra that acute nicotinic acid deficiency is characterized by the disappearance of F_2 excretion and a rise in F_1 excretion. As the disease becomes more chronic, the excretion of F_1 likewise tends to fall. The effect of nicotinic acid in reversing these changes is illustrated.

BIBLIOGRAPHY

1. Najjar, V. A., and Holt, L. E., Jr., The excretion of specific fluorescent substances in the urine in pellagra. *Science*, 1941, 93, 20.
2. Sydenstricker, V. P., Clinical manifestations of nicotinic acid and riboflavin deficiency (pellagra). *Ann. Int. Med.*, 1941, 14, 1499.
3. Najjar, V. A., and Wood, R. W., Presence of a hitherto unrecognized nicotinic acid derivative in urine. *Proc. Soc. Exper. Biol. and Med.*, 1940, 44, 386.

A STUDY OF THE REFLEX MECHANISM OF SWEATING IN THE HUMAN BEING; EFFECT OF ANESTHESIA AND SYMPATHECTOMY

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Many investigations have been carried out in an effort to determine the purpose and mechanism of sweating. Such important contributions as the separation of insensible from sensible perspiration, the part that evaporation from the skin plays in temperature control of the body (1), the demonstration of the "emotional" sweating of the palms, soles and axillae (2), and the absorption of ultraviolet radiation by sweat (3) have done much to explain the purposes of this important physiological function. However, the actual mechanism of sweating is still far from clear. It is generally accepted that the sweat glands are innervated by the sympathetic nervous system but their response to parasympathetic stimulants and depressants only is difficult to understand (4). In addition, it is uncertain whether the general sweat secretion which follows exposure to high environmental temperature is a reflex phenomenon resulting from stimulation of heat receptors in the skin, or from the effect of warmed blood bathing the heat center in the brain, or from both. Kuno (5), for example, concludes that sweating is a pure reflex phenomenon except when the temperature is extreme. Martin (6), on the other hand, concludes that the most effective stimulus for sweating is a "rise in the temperature of the blood supply to the brain." Best and Taylor (7) state that "the usual stimulus to sweat secretion is a rise in blood temperature which exerts its effect in two ways—directly upon the nervous centers, which is of more importance, and reflexly by stimulation of heat receptors in the skin." That sweating can occur by central stimulation has been well shown (8, 9).

Much of the work on which these conceptions were based was done on animals, and quantitative estimations were frequently omitted; consequently, it appeared that certain aspects of this problem might be more adequately studied on human beings. If various parts of the sweating

mechanism could be investigated separately, namely the sweat glands themselves, the motor sympathetic fibers and the sensory temperature fibers, it might be possible to determine whether the nervous reflex arc in whole or in part is essential for sweating in the human being.

METHOD AND APPARATUS

Of the various ways available for indicating qualitative activity of the sweat glands, a modification of the silver nitrate method was found most useful for our purposes (10). This test depends on the reduction of silver chloride by ultraviolet light. The area chosen for examination was carefully cleaned by soap, water, and alcohol. A white paper was then firmly pressed against this region for one minute. The paper was then removed, and ten per cent silver nitrate floated over it. After exposure to the sun or an ultraviolet lamp, if the sweat glands were active, small brown dots of reduced silver nitrate appeared on the paper which varied in number and intensity depending on the degree of sweat-gland activity. If the glands were inactive, no dots appeared.

The quantitative estimation of both sensible and insensible perspiration was determined as follows: Room air was sucked through a bottle of sulphuric acid and two large bottles of calcium chloride, by means of a water aspirator. Several preliminary tests indicated that the room air was in this way freed of moisture. The air then passed through an inverted glass cup with two side arms which was snugly applied and sealed by vacuum wax to the skin area to be tested. One side arm of the cup was attached by tubing to a weighed U-tube filled with fresh calcium chloride. Any increase in weight of this U-tube was a result of moisture added to the air from the surface of skin under the cup. The degree of negative pressure was determined by a mercury manometer which likewise checked the air-tightness of the apparatus. The negative pressure at times caused some discomfort to the subject, but checks against a positive pressure system showed that no excess water vapor was obtained in this way. The air flow was controlled and determined by a gasometer and the most satisfactory rate was found to be between 3 and 5 liters per minute.

In each experiment dry air was run through the apparatus for some time to insure the elimination of any retained moisture. The forehead and arm were chosen as the most desirable sites for the quantitative measurements as sweat glands are abundant in these regions and

no emotional sweating occurs in them except when psychic stimulation is very marked (11). The experiments were divided into exact ten-minute periods, and the U-tube was weighed and changed at the end of each of these. Control periods always preceded the heating periods. The presence or absence of sweat gland activity before the heat periods was confirmed by the silver nitrate test.

In those experiments in which heat was applied to an extremity, a radiant heater was used. This heater consists of a cage, open at both ends, containing a battery of eight electric light bulbs. The extremity to be tested was inserted into one of the open ends and radiation to other parts of the body surface was prevented by a blanket. By varying the number of lighted bulbs the temperature was maintained at approximately 48° C.

(1) *Normal sweat response to dry heat applied to an extremity.* The first group of experiments was performed on healthy males in order to determine the quantitative output of moisture on some remote region (forehead or opposite extremity) during exposure of an upper extremity to dry heat. These experiments served as a control. Seven tests were performed, three of which are reported in Table I. As can readily be seen, a general pattern is evident in all individuals studied, although the total quantitative output of moisture from similar areas on the forehead of different individuals varies. Likewise the onset of sweating after exposure to heat is more rapid in some individuals than in others (J. B. and R. J.). Undoubtedly the humidity, environmental temperature, and individual characteristics play a part in these variations. But for one exception (I. B.) the sweat glands as indicated by the silver nitrate test were not secreting prior to the application of heat with the individual at rest. In summary, then, it may be said that on the application of heat to one arm the sweat glands on the forehead or opposite arm become active immediately or after a latent period (R. J.) and a progressive increase in output of moisture from the region studied follows.

(2) *Histological study of the sweat glands in a sympathectomized area.* Before the reflex mechanism of sweating can be studied, the condition of the glands themselves after separation from their motor nerve supply must be determined. Severing the sympathetic nerve supply to an extremity is a common surgical procedure. If denervation is complete, diminution or absence of sweat gland activity in the sympathectomized region occurs.

TABLE I

Normal response

J.B., Male, August 4, 1941. Cup on forehead. Room temperature 26.9-27.7° C.

Heat left arm			Heat right arm		
Period	Perspiration	Silver nitrate test (Fore-head)	Period	Perspiration	Silver nitrate test (Fore-head)
	grams per 120 sq. mm. per 10 minutes			grams per 120 sq. mm. per 10 minutes	
Heat 48° C.					
1st Control	0.0237	—	1st Control	0.0220	—
2nd Control	0.0231	±	2nd Control	0.0207	±
L-1	0.0338	+	R-1	0.0270	±
L-2	0.0432	++	R-2	0.0342	++
L-3	0.0436	+++	R-3	0.0288	++
L-4	0.0532	++++	R-4	0.0406	+++

I.B., Male, July 23, 1941. Cup on forehead. Room temperature 27.7-28.5° C.

Heat right arm			Heat left arm		
Period	Perspiration	Silver nitrate test (Fore-head)	Period	Perspiration	Silver nitrate test (Fore-head)
	grams per 120 sq. mm. per 10 minutes			grams per 120 sq. mm. per 10 minutes	
Heat 48° C.					
1st Control	0.0360	++	C ₁ -1	0.0265	—
2nd Control	0.0338	+++	C ₁ -2	0.0291	+
3rd Control	0.0285	+++			
R-1	0.0385	+++	L-1	0.0287	+
R-2	0.0409	++	L-2	0.0312	++
R-3	0.0450	++++	L-3	0.0320	++
R-4	0.0445	++++	L-4	0.0312	++

R.J., Male, July 14, 1941. Cup on forehead. Room temperature 25-25.8° C.

Heat left arm			Heat right arm		
Period	Perspiration	Silver nitrate test (Fore-head)	Period	Perspiration	Silver nitrate test (Fore-head)
	grams per 120 sq. mm. per 10 minutes			grams per 120 sq. mm. per 10 minutes	
Heat 48° C.					
C-1	0.0130	—	C ₁ -1	0.0132	—
C-2	0.0125	—	C ₁ -2	0.0110	±
L-1	0.0153	+	R-1	0.0132	±
L-2	0.0145	—	R-2	0.0137	++
L-3	0.0208	+++	R-3	0.0140	+++
L-4	0.0223	+++	R-4	0.0210	+++

This surgical procedure prevents motor impulses from reaching the sweat glands; that is, it removes the motor side of the reflex arc. The question to be determined is whether the anhydrosis results from removal of the motor fibers to the sweat glands or whether it results from some change in the sweat glands themselves. Biopsy of the skin

in regions to which the sympathetic nerves had been severed failed to show any alteration in the appearance of the glands, as compared to the appearance of the glands in normal regions to which the sympathetic nerve supply was intact.¹

(3) *Direct stimulation of sweat glands in sympathectomized area by mecholyl.* A response of denervated sweat glands to a sudorific drug would be further proof of their integrity. Two women with Raynaud's disease were studied—one with a cervical sympathectomy and the other (M. H.) with a cervical and lumbar sympathectomy. In the former, both sympathetic chains had been cut below the 3rd dorsal ganglion and the 2nd and 3rd intercostal nerves had been severed just proximal to their union to form the nerve trunks. In the second patient (M. H.) a similar operative procedure was performed in the dorsal region and, in addition, the 2nd and 3rd lumbar ganglia had been resected bilaterally. This resulted in a quadrilateral sympathectomy in the second case. The first patient had an absence of noticeable sweating on the face and upper extremities and the second, on the face and all four extremities. Intracutaneous injection in the sympathectomized arms of 0.1 cc. of mecholyl in various dilutions, containing as little as 25. micrograms per cc. of the drug, invariably resulted in activity of the sweat glands in the injected area as indicated by the silver nitrate test. Injection of 0.1 cc. of sterile water as a control solution resulted in no activity of the sweat glands. In one instance the test was done ten days and in the other one year after operation. Judging from the number and size of the dots obtained by the silver nitrate test, the response to mecholyl was slightly less vigorous in the patient studied one year after denervation.

(4) *Direct stimulation of sweat glands in sympathectomized area by heat.* Procedures 2 and 3 above demonstrated that sweat glands deprived of their sympathetic nerve supply appeared normal histologically and could respond to intracutaneous injections of mecholyl. It was therefore of interest to determine whether denervated sweat glands could respond to the local application of heat. Hot pads applied to the sympathectomized

extremities in patient M. H., with cervical and lumbar sympathectomy, resulted in activity of the sweat glands in these areas as indicated by the silver nitrate test. No rise in the rectal temperature resulted from this procedure. A similar response can be seen in the second experiment (Table II): the sweat glands became active on the heated arm in the third heat period in spite of the fact that the sympathetic nerves were severed to this extremity. No activity of the sweat glands on the unheated right arm could be detected:

(5) *An attempt to stimulate the sweat glands in a sympathectomized area remote from heated region.* Procedures 2, 3 and 4 indicated that the sweat glands separated from their sympathetic nerves not only appeared normal histologically, but still responded to intracutaneous injections of a sudorific drug (mecholyl) and the local application of dry heat. The next question to be determined was whether these denervated glands could

TABLE II

Case with cervical and lumbar sympathectomy
M.H., Female, July 31, 1941. Cup on forehead. Heat applied to right arm. Room temperature 26.6–26.7° C.

Period	Perspiration	Silver nitrate test (Forehead)
	grams per 120 sq. mm. per 10 minutes	
Heat 48° C.		
C-1	0.0255	—
C-2	0.0177	—
R-1	0.0209	—
R-2	0.0185	—*
R-3	0.0200	—*
R-4	0.0212	—*

* Torso dripping wet.

M.H., Female, June 26, 1941. Cup on right arm. Heat applied to left arm. Room temperature 27.3–27.5° C.

Period	Perspiration	Silver nitrate test	
		Right arm	Left arm
	grams per 120 sq. mm. per 10 minutes		
Heat 48° C.			
C-1	0.0313	—	—
C-2	0.0276	—	—
L-1	0.0279	—	—
L-2	0.0274	—	—
L-3	0.0268	—	++
L-4	0.0292	—	+++*
L-5	0.0283	—	+++*
L-6	0.0293	±	++++*

* Torso dripping wet.

¹ We wish to thank Dr. Kornel Terplan, Professor of Pathology, University of Buffalo, for securing and examining these specimens for us.

respond to heat applied to a remote area having normal sensory innervation. Dry heat by means of the electric heater was applied to an arm of M. H., the patient referred to in procedure 4 above with cervical and lumbar sympathectomy. This operation, as described above, would prevent sympathetic impulses from reaching the areas to be studied. Likewise the heated region lacked sympathetic innervation, but the sensory nerves were intact. After a latent period profuse sweating appeared on the torso to which the sympathetic nerve supply was intact, but there was no significant increase in the amount of moisture obtained from the forehead or unheated arm to both of which the sympathetic nerves had been severed. These results are shown in Table II.

(6) *An attempt to stimulate the sweat glands from a remote area lacking heat sensation.* It appeared from the preceding experiment that sweat glands deprived of their sympathetic nerve supply would not secrete in response to heat applied to a distant region (the arm) possessing normal intact sensory innervation. The heat applied was sufficient to cause remote sweating in normal areas. The experiment to be described below was to discover whether sweat glands with an intact sympathetic nerve supply would respond to heat applied to another region, the sensory nerves of which had been destroyed. The patient M. L. had absence of pain and temperature sensation on his right side following an injury. On the application of dry heat to the anesthetic right arm, by the method described above, the silver nitrate test applied to the forehead showed a positive reaction and an essentially normal quantitative sweat response was produced on this remote part of the body (Table III). (Compare with normals in Table I.) Such significant increases in the amount of sweating from the forehead were always obtained in repeated experiments and, in addition, gross sweating was usually seen on the face, torso and legs.

(7) *An attempt to stimulate the sweat glands in a remote area by heating an anesthetized extremity.* The experiment just described had significance only if there was complete absence of sensory nerves from the heated extremity. Consequently, it was thought wise to duplicate it on another individual in whom the interruption of

TABLE III

Lack of sensation on right side

M.L., Male, July 21, 1941. Cup on forehead. Room temperature 25.2-25.5° C.

Heat normal left arm			Heat diseased right arm		
Period	Perspiration	Silver nitrate test (Forehead)	Period	Perspiration	Silver nitrate test (Forehead)
	grams per 120 sq. mm. per 10 minutes			grams per 120 sq. mm. per 10 minutes	
Heat 48° C.					
C-1	0.0176	-	C ₁ -1	0.0199	±
C-2	0.0153	-	C ₁ -2	0.0179	±
L-1	0.0145	-	R-1	0.0174	±
L-2	0.0149	+	R-2	0.0236	±
L-3	0.0223	++++	R-3	0.0470	++++
L-4	0.0341	++++	R-4	0.0267	++++

M.L., Male, June 20, 1941. Cup on forehead. Room temperature 23.2-25.5° C.

Heat diseased right arm			Heat normal left arm		
Period	Perspiration	Silver nitrate test (Forehead)	Period	Perspiration	Silver nitrate test (Forehead)
	grams per 120 sq. mm. per 10 minutes			grams per 120 sq. mm. per 10 minutes	
Heat 48° C.					
C-1	0.0209	+	C ₁ -1	0.0412	-
C-2	0.0226	+	C ₁ -2		
R-1	0.0377	-	L-1	0.0424	++
R-2	0.0426	++	L-2	0.0522	++
R-3	0.0530	+++	L-3	0.0621	++++
R-4	0.0571	+++	L-4	0.0730	++++

the sensory nerves from the heated extremity was certain. The patient (M. A.) was a colored woman, probably suffering from Raynaud's disease but who had been free from symptoms for a year although she had received no treatment during that time. A brachial plexus block was established on the left side²: miosis, ptosis and enophthalmos of the left eye were present, but there was no anhidrosis. No sensation whatsoever was present in the left arm during the course of the experiment. Heating the anesthetized arm resulted in copious sweating on the forehead, a response greater than the control period before the block was performed (Table IV). The heated arm appeared perfectly dry and no evidence of sweat gland activity could be detected by the silver nitrate test.

² We wish to thank Dr. Paul Searles, Professor of Anesthesia, University of Buffalo, for this difficult and expert technique.

TABLE IV

Left brachial plexus blocked

M.A., Female, August 11, 1941. Cup on right side of forehead. Room temperature 22.6–23.8° C.

Heat left arm					
Normal response			After block of left brachial plexus		
Period	Perspiration	(Forehead) AgNO ₃ test	Period	Perspiration	(Forehead) AgNO ₃ test
C-1	0.0188	—	C ₁ -1	0.0210	—
C-2	0.0173	—	C ₁ -2		
L-1	0.0221	+	L ₁ -1	0.0308	++
L-2	0.0258	++	L ₁ -2	0.0547	+++
L-3	0.0359	+++	L ₁ -3	0.0570	Gross sweating visible (—) Left arm
L-4	0.0385	+++	L ₁ -4	0.0686	

DISCUSSION

It is well known that following sympathectomy apparent anhidrosis of the region affected commonly occurs (12). Heating a normal extremity for sufficient length of time and with sufficient intensity to cause general sweating in a normal person failed to produce a quantitative increase in moisture output in remote sympathectomized regions in the experiments described. Thus it appears that the sympathetic innervation to the sweat glands or the motor side of the reflex arc must be intact to obtain a normal sweat response to heat applied to a remote region. That the sweat glands themselves are not at fault is well shown by their response to intracutaneous injections of mecholyl and to excessive local heat stimulation in areas to which the sympathetic nerves have been severed. Lewis and Landis (13) have similarly demonstrated that sweating can be provoked by pilocarpine. That the response of the sweat glands in sympathectomized areas to mecholyl and heat is not dependent on any nervous mechanism is strongly supported by the work of Bickford (14) who was unable to obtain any sweating response to a faradic stimulus in an arm sympathectomized two years previously. Additional evidence that the glands themselves are normal is afforded by histological study.

The normal or excessive response of the sweat glands in remote areas to heat applied to a region deprived of its sensory nerve supply indicates that the sensory side of the reflex arc is unnecessary for a normal sweating response. This was well

shown in two individuals (M. L. and M. A.). This suggests that the heated blood acts directly on the central nervous system and results in a generalized sweating response except where sympathetic fibers supplying some particular area have been severed. Our subject M. A. showed a greater sweating response on the forehead on application of heat to the arm following anesthesia of the brachial plexus than in the control period. A possible explanation for this increase might be the vasodilatation of the arm vessels due to paralysis of the vasomotor fibers, with a greater amount of blood consequently exposed to the heat than during the control period. Thus more heated blood was carried to the central nervous system with a greater sweating response. However, some hormonal effect as the cause of sweating cannot be ruled out. That local stimulation of the sweat glands by the heated blood did not occur is shown by the negative silver nitrate test on the treated arm.

The failure of dry heat, when applied to the anesthetized arm, to cause activity of the sweat glands on the heated arm in this patient is in direct contrast to the activity of the sweat glands obtained in M. H. (Table II, Experiment II). A possible explanation for this contradiction is that in the patient with the brachial plexus block the sweat glands have not yet become sensitized, the block being performed just prior to the heating; whereas in the patient M. H., the sympathectomy was performed one year previously. Such sensitization is a common phenomenon in all tissues innervated by the sympathetic nervous system.

CONCLUSIONS

No histological change can be seen in sweat glands deprived of their sympathetic nerve supply.

The sweat glands themselves, in spite of severance of their sympathetic nerve supply, are still able to respond to intracutaneous injections of mecholyl and excessive local stimulation by heat.

In a human being the sympathetic fibers, or motor side of the reflex arc, must be intact for a normal sweating response to occur in a region remote from the heated area.

In spite of the absence of sensation in a heated region, a normal sweat response can still occur on other areas. The sensory side of the reflex

consequently unnecessary for a normal sweating response to occur following the application of heat. This suggests that heated blood acting on the central nervous system is the cause of generalized sweating. Whether the heated blood acts directly on the so-called heat centers or by liberating some hormone which in turn stimulates the centers is still uncertain.

The authors wish to express their gratitude to Dr. Fred R. Griffith, Jr., Professor of Physiology, who made many helpful suggestions in the carrying out of this work. We also wish to thank Dr. J. S. Regan and Dr. Gilbert Beck who furnished us the patients for study. The Department of Physiotherapy of the Buffalo General Hospital was very helpful in the loan of some of its apparatus and assistance in general.

BIBLIOGRAPHY

1. Dubois, E. F., *Basal Metabolism in Health and Disease*. Lea and Febiger, Philadelphia, 1936, p. 445.
2. Kuno, Y., *The Physiology of Human Perspiration*. Churchill, Ltd., London, 1934, p. 129.
3. Crew, W. H., and Whittle, C. H., On the absorption of ultra-violet radiation by human sweat. *J. Physiol.*, 1938, 93, 335.
4. List, C. F., and Peet, M. M., Sweat secretion in man. *Arch. Neur. and Psychiat.*, 1938, 40, 269.
5. Kuno, Y., *The Physiology of Human Perspiration*. Churchill, Ltd., London, 1934, p. 205.
6. Martin, C. J., Thermal adjustments of man and animals to external conditions. *Lancet*, 1930, 2, 673.
7. Best, C. H., and Taylor, N. B., *Physiological Basis of Medical Practice*. Williams and Wilkins, Baltimore, 1939, p. 1007.
8. Martin, C. J., Thermal adjustments of man and animals to external conditions. *Lancet*, 1930, 2, 561.
9. Cushing, H., *Papers relating to the pituitary body, hypothalamus and parasympathic nervous system*. Charles C. Thomas, Springfield, Ill., 1932, p. 55.
10. Aubert, Lyon Medical, 1874. Cited by Luciani, L., *Human Physiology*. MacMillan and Co., Ltd., London, 1913, p. 487.
11. Kuno, Y., *The Physiology of Human Perspiration*. Churchill, Ltd., London, 1934, p. 130.
12. Guttman, L., The distribution of disturbances of sweat secretion after extirpation of certain sympathetic cervical ganglia in man. *J. Anat.*, 1940, 74, 537.
13. Lewis, T., and Landis, E. M., Some physiological effects of sympathetic ganglionectomy in the human being and its effect in a case of Raynaud's malady. *Heart*, 1930, 15, 151.
14. Bickford, R. G., The mechanism of local sweating in response to faradism. *Clin. Sc.*, 1938, 3, 337.

THE METABOLISM OF AMINO ACIDS IN DIABETES MELLITUS

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(Received for publication November 13, 1941)

The amino acid content of fasting human blood appears to be quite constant. Extensive surveys (1) have revealed only a few diseases in which the blood amino acid level is modified significantly. Acute yellow atrophy of the liver is associated with a rise in amino acids in the blood, whereas hypoglycemia has been reported in patients with nephrosis and in pneumococcal pneumonia (2).

Concerning the fasting amino acid level in diabetes mellitus there is little agreement. Several investigators (1a, 3) have observed elevated amino acid levels in a few of their diabetic subjects. Greene, Sandiford and Ross (1b) studied 116 diabetics and observed that the average amino acid level was within normal limits. No accurate data on the condition or treatment of these patients were given. On the other hand, a high blood amino acid content has been reported in dogs with experimentally induced diabetes (4).

Luck, Morrison and Wilbur (5) observed that insulin lowered the amino acid content of blood in both experimental animals and normal human subjects. These results have been confirmed by several investigators (6) using the Folin colorimetric method and by Farr and Alpert (7) using Van Slyke and MacFadyen's manometric ninhydrin method (8). Luck also studied two diabetic patients under insulin treatment and observed low normal fasting amino acid levels.

The lack of agreement of existing data on the blood amino acid content in human diabetes mellitus, together with the paucity of data on the clinical condition and therapy of the patients, suggested the present study.

METHODS

1. Choice of subjects. A group of twelve severe untreated diabetic patients was studied. All of these patients were admitted to the Johns Hopkins Hospital primarily because of diabetes, although complications (alveolar abscess, chronic sinusitis, chronic infection of hand) were present in several instances. In four of the twelve patients, the CO_2 combining power was above 50 volumes per cent on admission. The other eight patients were in moderate to severe acidosis (Table I).

2. Method of study. In all cases, blood was drawn immediately after entry. In no case had nourishment been taken in the preceding three hours. Subsequent blood specimens were then drawn at various intervals during the course of therapy. The patients with severe acidosis were given hourly injections of insulin, with supplementary carbohydrate as indicated. Nitrogen balance studies were made on two patients.

In three of the patients, glycine (0.27 gram per kilo) was given intravenously and the effect on the blood and urinary amino acid, urea, and sugar levels was studied. From these data, the rate of deamination was estimated. These patients were studied subsequently during insulin treatment.

3. Chemical methods. The amino acid content of the blood plasma was determined by the manometric ninhydrin method of Van Slyke and MacFadyen (8b). This method is considered to be more specific than previous methods, and gives somewhat lower values. Normal limits, as given by Farr and MacFadyen (2a), are 3.6 to 5.4 mgm. calculated as nitrogen per 100 cc. of plasma. Most of the determinations were done in duplicate, and all determinations were checked with blank and known samples. In two cases, the results were compared with those of the colorimetric method of Folin (11). Analysis of urine for amino acid content was made by the manometric ninhydrin method. The low concentration of amino acids and the high concentration of substances which may give false reactions in urine make these values less significant than the blood determinations.

Since the manometric ninhydrin method depends on the measurement of evolved carbon dioxide, other reactions resulting in the production of carbon dioxide from diabetic plasma must be considered. Pre-existing carbon dioxide and bicarbonate are removed and thus cannot affect the result. The possibility of a slow evolution of carbon dioxide from some abnormal constituent of the blood was ruled out by performing the analysis with ninhydrin omitted. No carbon dioxide was produced under these conditions. The reaction of abnormal constituents with ninhydrin is also possible. This is ruled out as an important factor by the similarity of results obtained by the manometric ninhydrin method and by Folin's colorimetric method.

The urea content of blood and urine was determined by the urease method and the non-protein nitrogen by the micro-Kjeldahl.

RESULTS

1. Plasma amino acid levels in untreated diabetes

All twelve of the untreated diabetic patients had a plasma amino acid content exceeding the

TABLE I

Chemical changes following insulin therapy in diabetic patients

Case number	Time after admission	Blood					Urine	
		Plasma amino N	Urea N	NPN total	Sugar	Plasma CO ₂ combining power	Sugar	Acetone
1	0	19.8	16	42	182	55	4+	4+
	3 weeks	4.8	13	26	235	58	4+	0
2	0	17.3	41	80	768	19	4+	4+
	1 day	5.5		46	60	59	0	trace
	2 days	4.5	21	40	420	65	4+	0
3	0	15.0	23	58	396	28	4+	4+
	1 day	5.5	17	36	213	48	2+	trace
	2 days	5.8	15	29	200	43	2+	trace
	3 days	4.6			150	0	0	0
	5 days	4.9			200	51	2+	0
	7 days	5.6			490	58	4+	0
	10 days	5.0			192		trace	0
4	0	14.5	35	68	470	31	4+	3+
	1 day	7.2	23	46	105	60	0	trace
	8 days	5.1	14	30	207		1+	0
5	0	13.4	17	40	500	12	4+	4+
	1 day	8.1	10	26	226	43	4+	1+
	2 days	5.3	10		170	54	0	0
6	0	12.5	25	52	261	23	4+	4+
	2 days	5.1	12	27	225	64	3+	0
7	0	10.8	45	74	840	32	4+	4+
	1 day	5.8	32	62	96	53	0	1+
	2 days	4.7	20	40	220	47	1+	0
	4 days	5.5	13	28	260	58	2+	0
	6 days	5.4		24	179	55	trace	0
	15 days	5.1		30	135	60	0	0
8	0	10.3	16	36	300	43	4+	3+
	1 day	6.1			177	50	0	trace
	2 days	5.4	13	30	195	54	0	0
	3 days	5.3			195	58	0	0
9	0	9.6	14	34	436	55	4+	2+
	8 days	5.9	13	27	130		0	0
10	0	9.5	13	43	496	9	4+	4+
	1 day	5.0	10	28	149	56	0	trace
11	0	8.3	15	30	348	52	4+	4+
	12 days	5.9	14	25	142		0	0
12	0	7.5	17	34	230	60	4+	2+
	2 days	5.7			118		1+	trace
	3 days	5.5	12	28	132		trace	0
	5 days	5.3		26	105		0	0
	10 days	5.8			90		0	0

four cases, the fasting levels exceeded the normal fasting upper limit of 5.4 mgm. per 100 cc. The amino acid content of whole blood and of packed red blood cells was similarly increased, the erythrocytes maintaining a slightly higher level than the plasma. In two cases the amino acid levels obtained by the ninhydrin method were compared with determinations of amino acids by the colorimetric method of Folin. The results of the two methods were very similar. The colorimetric method yielded slightly lower values when the manometric ninhydrin results were very high, while at normal levels, the ninhydrin method gave slightly lower values.

2. Relation of blood amino acid content to the degree of acidosis or azotemia

The highest amino acid level was recorded in a patient with a normal plasma CO₂ combining power (Case 1). It is to be noted, however, that all of the patients in diabetic acidosis had a significantly elevated blood amino acid content. Strong tests for acetone and diacetic acid in the urine were always noted when the blood amino acid content was much above normal. There was no correlation between the blood content of amino acids and that of urea.

3. Amino acid content of urine

The loss of substances reacting as amino acids in the urine was much increased in patients with severe diabetes. This fact has been noted (9). Untreated diabetics excreted from four to ten times the normal quantity of amino acids in the urine (Figure 2).

4. Effect of insulin

The elevated amino acid content of untreated diabetics was restored to normal by insulin therapy (Table I). During the first few hours of intensive insulin therapy the decrease in blood amino acids roughly paralleled the reduction in blood sugar in a patient with severe acidosis (Figure 1). Within twelve to forty-eight hours, the plasma amino acid level attained normal or even subnormal values. From this point onward, the amino acid level was rather constant, provided that sufficient insulin and carbohydrate were given. A considerable dissociation between the twenty-four-hour curves of blood concentration of sugar

normal fasting level (Table I). In eight cases, the content was greater than 10 mgm. per 100 cc. This value probably exceeds the upper limit of even normal post-absorptive rise. In the other

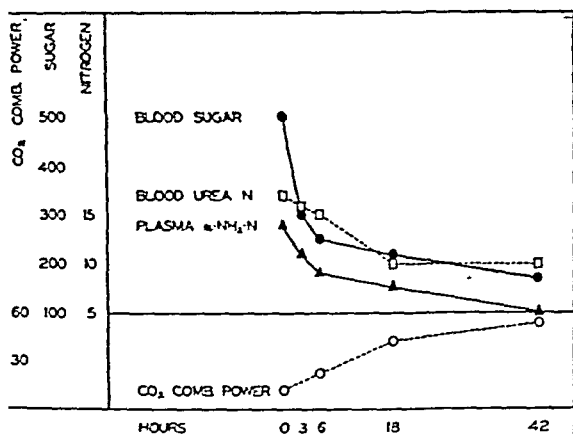


FIG. 1. CHANGES IN CONCENTRATION OF VARIOUS SUBSTANCES IN THE BLOOD DURING THE FIRST FORTY-TWO HOURS OF TREATMENT OF A PATIENT WITH SEVERE DIABETIC ACIDOSIS

There is a rough parallelism between the falling concentration curves of blood sugar and amino acids.

and amino acid was observed during the period of adjustment of diet and insulin, since there were frequently proportionately larger and more rapid variations in the blood sugar. Hypoaminoacidemia (below 3.6 mgm. N per cent) was rarely seen following large doses of insulin. Whether hypoglycemia occurred at the same time depended on the carbohydrate intake.

The effect of insulin on the increased renal excretion of nitrogen in uncontrolled diabetes is well known. In the two patients in whom nitrogen excretion studies were made, the fall in the blood amino acid level occurred somewhat more abruptly than the diminution of renal nitrogen excretion. The disappearance of acetone and diacetic acid from the urine also lagged behind the fall of blood amino acid.

5. Tolerance and utilization of intravenously injected glycine

The high blood level of amino acids in uncontrolled diabetes presumably arises from some disturbance in production or utilization. In order to study certain aspects of this problem, glycine (0.27 gram per kilo) was injected intravenously in 10 per cent solution over a period of forty-five minutes and the blood amino acid, urea, and sugar were followed for the subsequent three-hour period. Urine was collected during a control period before the test, and hourly during the test. The

patients were given the glycine before insulin therapy had been instituted. The same routine was repeated subsequently in two of the patients after they were well balanced on protamine zinc insulin, the test being performed in the early morning, ten hours following the last nourishment, and nearly twenty-four hours after the last insulin injection. Similar tests were performed on normal subjects. Data are presented graphically in Figure 2.

It is evident that the uncontrolled diabetic patient responded to the injection of a moderate amount of glycine in a considerably different fashion from a normal subject, or from an insulin-treated diabetic. In untreated severe diabetes, the plasma amino acid level started from, and rose to, higher levels. Loss of amino acid in the urine was increased. A large rise in blood urea was not observed. There was a considerable increase in urea excretion, however, which during the course of three hours was equivalent to a large proportion of the nitrogen of the injected glycine. There was also a significant rise in blood sugar and in urinary sugar excretion. In contrast, the insulin-treated diabetic patient and the normal individual show much smaller changes than the severe untreated diabetic, although the direction of each reaction appears to be the same.

DISCUSSION

These observations indicate that the plasma amino acid content is greatly elevated in untreated severe diabetes. Concomitantly, the urinary output of amino acids is considerably increased. The increased blood amino acid level does not appear to be due to impairment of excretory function of the kidney, since the output of amino acid is increased above the normal. In addition, there is no correlation between urea retention and the increase of amino acid in the blood. The degree of ketonemia does not affect the plasma amino acid content significantly.

Insulin has a rapid and profound effect in lowering the elevated blood amino acid level. During the first few hours of insulin treatment, the falling curves of blood amino acids and sugar are roughly parallel. When carbohydrate is administered, however, the parallelism ceases. The plasma amino acid content remains rather con-

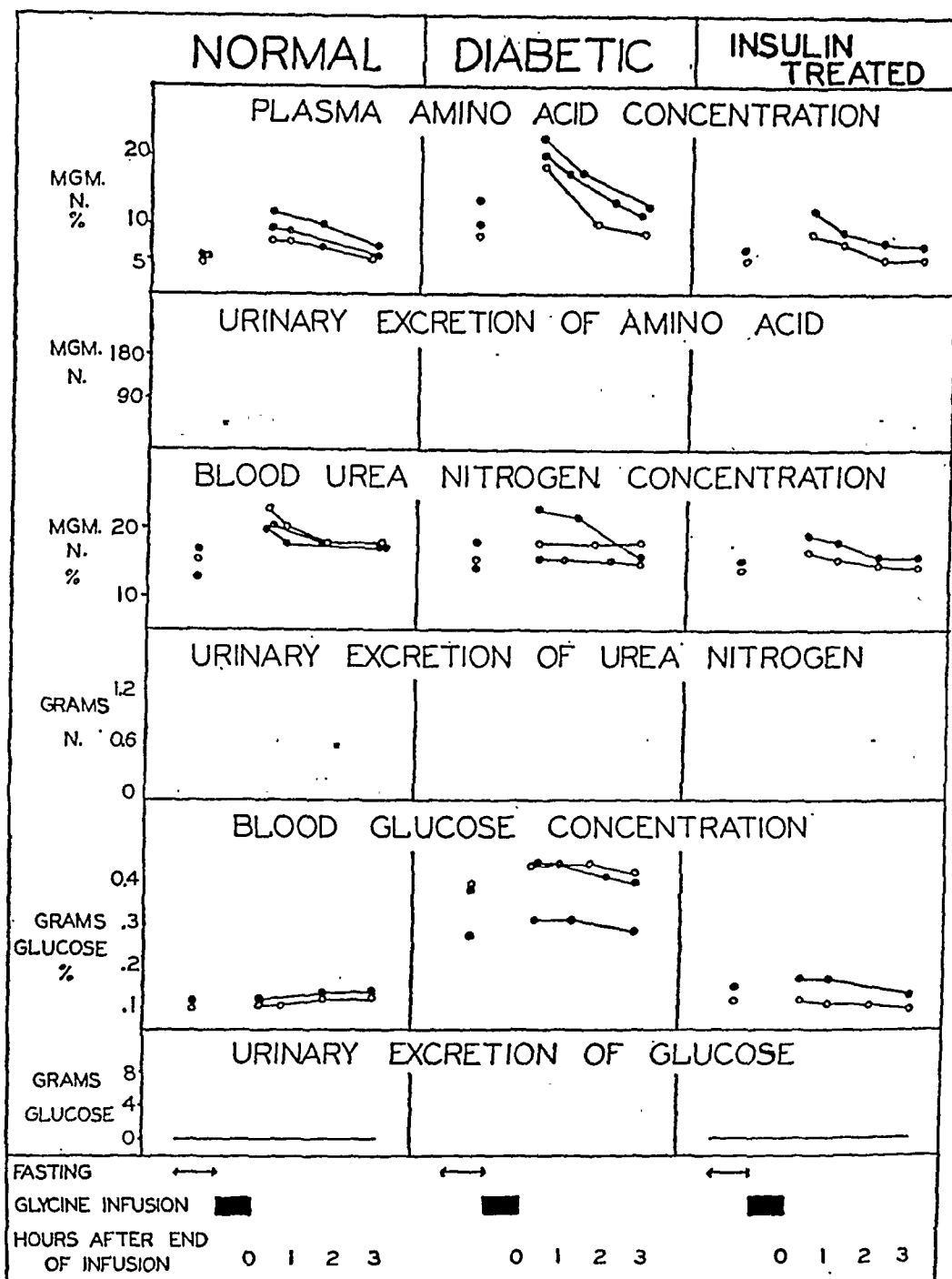


FIG. 2. GRAPHIC SUMMARY OF RESULTS OF INTRAVENOUS AMINO ACID TOLERANCE CURVES IN NORMAL SUBJECTS AND IN SEVERE DIABETIC PATIENTS BEFORE AND AFTER INSULIN THERAPY

stant under insulin therapy, and the fasting and post-absorptive levels are well within normal limits. In contrast, during the same period the blood sugar level may show wide swings. A similar effect was observed by Luck and Morse (10) in normal humans when the maintenance of a normal blood sugar did not prevent or diminish appreciably the lowering of the normal blood amino acid content by large doses of insulin.

When insulin is administered to a severe diabetic, there is a rapid diminution in renal excretion of urea. This occurred a few hours after the fall in amino acids in the two cases studied. There is also a difference in the handling of intravenously injected glycine by diabetic patients before and after insulin therapy. Plasma amino acids reached higher levels in the untreated patients, and more glycine was lost in the uri-

(Figure 2). There is also an unusually large increase in urea nitrogen excretion in the untreated diabetics following glycine injection. The changes in blood and urine urea and glucose (Figure 2) superficially suggest an increased rate of deamination and gluconeogenesis, but with the limited data, it is not possible to be certain. One can be certain, however, that in the severe untreated diabetic, the rate of urea production from amino acids is at least equal to normal.

If there is no decrease in the rate of deamination of amino acids in severe untreated diabetes, the increased concentration of plasma amino acids would appear to be due to an increase in the rate of production or liberation of amino acids. Conversely, insulin would appear to diminish the rate of production or liberation of amino acids, since insulin lowers the concentration of plasma amino acids at a time when the production of urea is diminishing. A similar conclusion on the action of insulin was reached by Mirsky (6a) in his studies on nephrectomized non-diabetic animals.

SUMMARY AND CONCLUSIONS

1. Twelve patients with severe untreated diabetes mellitus had high fasting plasma amino acid levels.
2. The high plasma levels were accompanied by increased urinary excretion of amino acids, and could not be correlated with urea retention.
3. The administration of insulin caused a rapid return of blood levels to normal. On continued insulin therapy, the plasma amino acid was maintained at normal levels, despite the fluctuation of the blood sugar.
4. Evidence suggests that in severe untreated diabetes mellitus, there is an increase in the rate of production or liberation of amino acids, and that the administration of insulin is followed by the return to a more normal rate.

The author is greatly indebted to Doctor George W. Thorn for helpful suggestions and criticism of this work.

BIBLIOGRAPHY

- 1a. Desqueyroux, J., Recherches cliniques sur l'aminocidémie. *Ann. de méd.*, 1923, 13, 20.

- b. Greene, C. H., Sandiford, K., and Ross, H., The amino acid content of the blood in normal and pathologic conditions. *J. Biol. Chem.*, 1924, 58, 845.
- c. Feinblatt, H. M., and Shapiro, I., The amino acid content of blood in various pathologic conditions. *Arch. Int. Med.*, 1924, 34, 690.
- d. Edgar, S. H., The amino acid content of the blood of children in health and disease. *Biochem. J.*, 1928, 22, 168.
- 2a. Farr, L. E., and MacFadyen, D. A., Hypoaminoacidemia in children with nephrotic crises. *Am. J. Dis. Child.*, 1940, 59, 782.
- b. Farr, L. E., MacLeod, C. M., Fletcher, P. H., Emerson, K., Mirick, G. S., and Curnen, E. C., Hypoaminoacidemia in patients with pneumococcal pneumonia. *Proc. Soc. Exper. Biol. and Med.*, 1940, 44, 290.
3. Wolpe, G., Ueber Aminosäuren im Blutserum, im Liquor Cerebrospinalis, und in Punktionsflüssigkeiten. *München. med. Wchnschr.*, 1924, 71, 363.
4. Okada, S., and Hayashi, T., Studies on the amino acid nitrogen content of the blood. *J. Biol. Chem.*, 1922, 51, 121.
5. Luck, J. M., Morrison, G., and Wilbur, L. F., The effect of insulin on the amino acid content of blood. *J. Biol. Chem.*, 1928, 77, 151.
- 6a. Mirsky, I. A., The influence of insulin on the protein metabolism of nephrectomized dogs. *Am. J. Physiol.*, 1938, 124, 569.
- b. Kerr, S. E., and Krikorian, V. H., The effect of insulin on the distribution of non-protein nitrogen in the blood. *J. Biol. Chem.*, 1929, 81, 421.
- c. Powers, H. H., and Reis, F., The effect of insulin on amino acid and urea nitrogen in laked and unlaked blood. *J. Biol. Chem.*, 1933, 101, 523.
7. Farr, L. E., and Alpert, L. K., The effect of endocrine extracts on the amino acids in the blood, with incidental findings on the blood sugar and urea. *Am. J. Physiol.*, 1940, 128, 772.
- 8a. Van Slyke, D. D., and Dillon, R. T., Gasometric determination of carboxyl groups in amino acid. *Compt. rend. Lab. Carlsberg, series chim.*, 1938, 22, 480.
- b. Personal communication of plasma method to be published by Van Slyke and MacFadyen.
- 9a. Galambos, A., and Tausz, B., Ueber Eiweissstoffwechsel bei Diabetes Mellitus. *Z. Klin. Med.*, 1913, 77, 14.
- b. Labbé, M., and Bith, H., L'aminocidurie Pathologique. *Presse Med.*, 1913, 21, 698.
10. Luck, J. M., and Morse, S. W., Effects of insulin and adrenalin on the amino acid content of blood. *Biochem. J.*, 1933, 27, 1648.
11. Folin, O., A system of blood analysis. Supplement III. A new colorimetric method for the determination of the amino acid nitrogen in blood. *J. Biol. Chem.*, 1922, 51, 393.

CHEMOTHERAPY OF PNEUMOCOCCIC MENINGITIS WITH SPECIAL REFERENCE TO SULFATHIAZOLE

By FRANK B. COOPER, PAUL GROSS, AND MARION L. HAGAN

(From the Western Pennsylvania Hospital Institute of Pathology, Pittsburgh)

(Received for publication November 15, 1941)

Because the sulfathiazole content of the brain and cerebrospinal fluid is admittedly low compared to that of the blood during medication, it has recently been claimed, in the absence of pertinent experimental or clinical data, that sulfathiazole is of little or no value in treating bacterial meningitis (1 to 4). Although it is not the purpose of this paper to advise the continued use of sulfathiazole in clinical cases of meningitis or to recommend to the clinician the use of any one of the sulfonamides in preference to the others, the experimental data presented and the clinical cases collected from the literature show that the above claim (1 to 4), as well as certain other disseminated opinions, require modification.

METHOD

Two hundred rats of approximately 200 grams weight were lightly anesthetized with ether and infected intracranially with approximately one fatal dose of Type II pneumococci. The animals were divided into five groups: one served as control; the remaining four groups were treated orally with 100 mgm. of the various drugs suspended in 0.5 cc. of 15 per cent gum acacia 6, 22, 46, and 96 hours after infection. One group was treated with sulfanilamide, one with sulfapyridine, one with sulfathiazole, and the last with sulfamethylthiazole (Table I). All fatalities were autopsied and those showing in-

sufficient pneumococcal infection to explain death were not included in the experiment.

To ascertain the relationship between the concentration of sulfathiazole in the blood and brain, 45 similarly infected rats were given 100 mgm. of drug orally, followed by 100 mgm. 8 hours and 24 hours later. At the intervals indicated in Figure 1 a number of rats were lightly etherized and 0.5 cc. of blood was withdrawn by cardiac puncture for analysis. No rat was bled more frequently than once during 24 hours. Rats were sacrificed after the 25-, 26-, 27-, 28-, 29-, and 30-hour bleedings and the drug content of the brain was determined. The analytical method employed was a modification of that of Bratton and Marshall (5) and has been shown statistically to give significantly higher recovery of sulfathiazole from blood, especially at 1:20 dilution (6).

RESULTS

Reference to Table I shows a 21-day survival of 18 per cent of the control group, compared to 84 per cent of the sulfanilamide group, 87 per cent of the sulfapyridine group, 79 per cent of the sulfathiazole group and 78 per cent of the sulfamethylthiazole group.

The concentration of sulfathiazole in the blood of rats receiving comparable treatment lay along a fairly smooth curve during the 7 hours following the first treatment (Figure 1). During the 6 hours following the 24-hour treatment, the con-

TABLE I

Type II pneumococcal meningitis in rats

Treatment	Number of rats	Number of deaths daily during 21 days									Number of survivors	Per cent survivors
		1	2	3	4	5	6	7	8	9-21		
None.....	40	16	13	2	1				1		7	18
Sulfanilamide (1).....	38				1	2	2			1	32	84
Sulfapyridine (2).....	39				3	1	1				34	87
Sulfathiazole (3).....	34				1	2	3			1	27	79
Sulfamethylthiazole (3).....	37				2	2	2	1		1	29	78

Infection: 0.1 cc. of a 10^{-6} broth dilution of an 18-hour broth culture (Binda, Type II) intracranially (approximately one fatal dose).

Treatment: 100 mgm. of drug suspended in 0.5 cc. of 15 per cent gum acacia orally 6, 22, 46 and 96 hours after infection (total 400 mgm.).

Drugs were synthesized and donated by (1) Monsanto Chemical Company, St. Louis, Missouri; (2) E. R. Squibb and Sons, New York City; and (3) The Maltbie Chemical Company, Newark, N. J.

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None.....	40	16	13	2	1				1		7	18
Sulfanilamide (1).....	38				1	2	2				32	84
Sulfapyridine (2).....	39				3	1	1			1	34	87
Sulfathiazole (3).....	34				1	2	3			1	27	79
Sulfamethylthiazole (3).....	37				2	2	2	1		1	29	78

Infection: 0.1 cc. of a 10^{-6} broth dilution of an 18-hour broth culture (Binda, Type II) intracranially (approximately one fatal dose).

Treatment: 100 mgm. of drug suspended in 0.5 cc. of 15 per cent gum acacia orally 6, 22, 46 and 96 hours after infection (total 400 mgm.).

Drugs were synthesized and donated by (1) Monsanto Chemical Company, St. Louis, Missouri; (2) E. R. Squibb and Sons, New York City; and (3) The Maltbie Chemical Company, Newark, N. J.

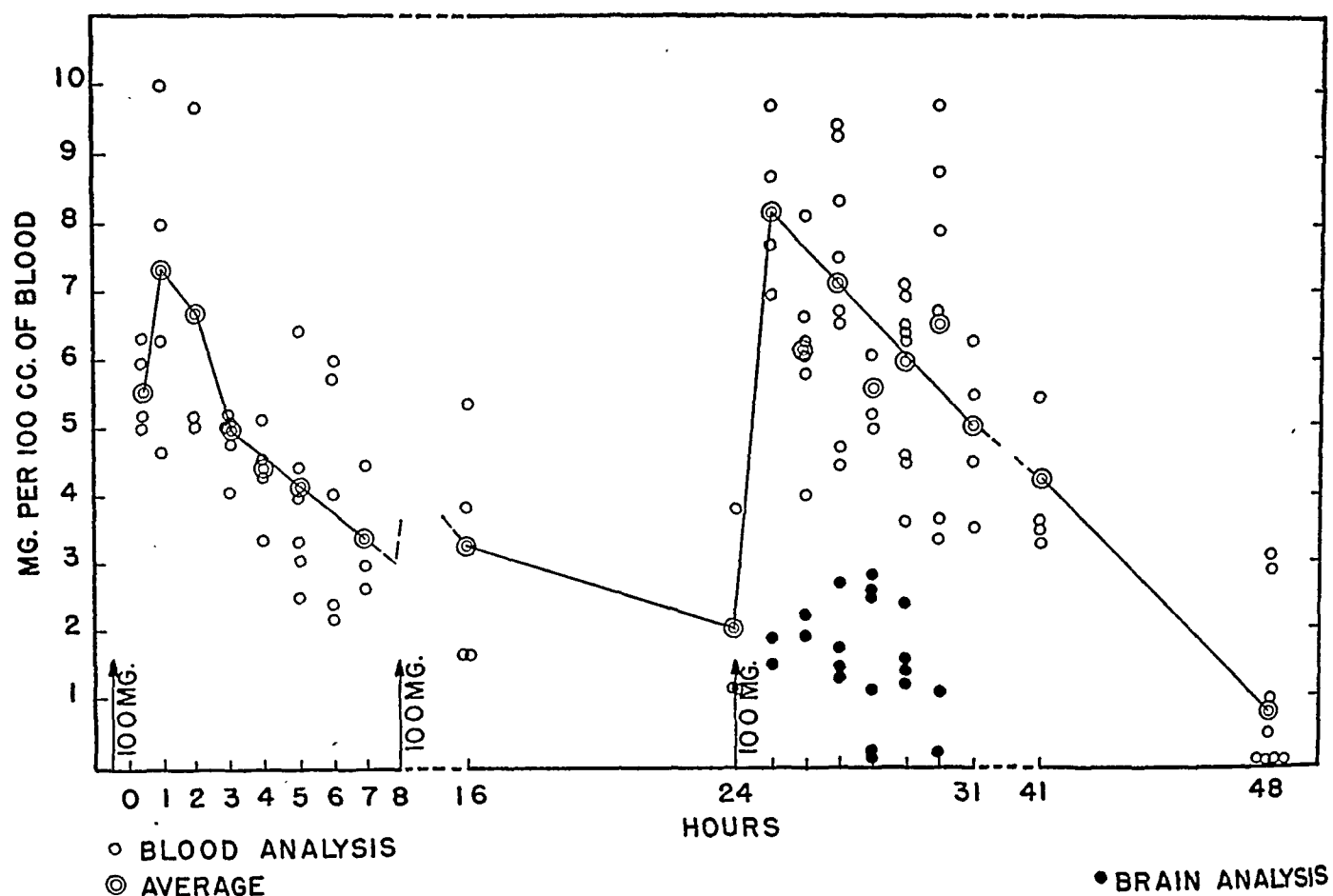


FIG. 1. SULFATHIAZOLE CONCENTRATION IN BLOOD AND BRAIN OF MENINGITIC RATS

centrations were higher and the variations greater. The brains of rats killed during the interval when blood concentrations varied from 3.5 to 10 mgm. per cent, and averaged 5 to 6 mgm. per cent, showed drug concentrations which varied from traces to 3 mgm. per cent and averaged approximately 1.6 mgm. per cent.

DISCUSSION

In a previous report (7) it was demonstrated that pneumococcic meningitis in rats is at least as severe as in man. In untreated animals, the occurrence of empyema of the cerebral ventricles and of the central canal of the cord was not uncommon. Extensions of the purulent meningitis into the nervous parenchyma occurred and were often associated with abscesses. Proof that the infection was not eliminated before lesions had become established was seen in the disturbances in gait and equilibrium in treated animals which recovered. Anatomic proof was obtained in the histologic sections of the brain and cord of these animals (8). The significant findings included

thickened meninges with lymphocytic infiltration and vascular scars in the cerebral cortex with clusters of phagocytic cells, hemosiderin deposits and foci of calcification.

The infecting dose was purposely kept low so that successful treatment with the various drugs remained below the level known to cause kidney damage and its sequelae in rats (9 to 11).

Because the greatest difference in group survival was 9 per cent, the results were tested for significance by the χ^2 method. Taking the probability value of 0.05 as the criterion of significance, it was found that the differences observed among the treated groups were without statistical significance.

This proof that sulfanilamide, sulfapyridine, and sulfathiazole are equally effective in experimental pneumococcic meningitis of rats infected with the Binda Type II strain raises the interesting question of the relative importance of a high concentration of free drug in the cerebrospinal fluid for successful chemotherapy of meningitis. The blood of infected rats which received 300

mgm. of sulfathiazole in 24 hours (Figure 1), averaged 5 to 6 mgm. per cent of free drug, while the brains averaged 1.6 mgm. per cent and occasionally contained considerably less than 1 mgm. per cent.

Chemotherapeutic results of clinical meningitis as collected from the literature

The successful treatment with sulfathiazole of experimental pneumococcic meningitis in rats has its counterpart in the treatment of clinical meningitis. Table II lists not only 4 typed cases of pneumococcic meningitis but one case of streptococcic meningitis, 9 cases of staphylococcic meningitis, and 125 cases of meningococcic meningitis, the cure of which was attributed to therapy with sulfathiazole or its sodium salt.

The evaluation of the clinical efficacy of the various sulfanilamide drugs has resulted in rules which, on the whole, seem to be workable. However, several opinions disseminated in the medical literature appear, on the basis of subsequent clinical experience, to require modification. A case in point was the early and persistent assertions, frequently based on none too critical animal experiments, that sulfanilamide was of little or no value in pneumococcic infections (12 to 17) or was of value in treating only Type III pneumococcic infections (18 to 20). Disregard of these asser-

TABLE II

Pneumococcic, streptococcic, staphylococcic, and meningococcic meningitis recoveries attributed to sulfathiazole

Investigator	Treatment	Infection	Number of recoveries
Spink and Hansen	ST*+S†	Type III pneumococcus	1
Myers, Robb and Clapper	ST+SMT‡	Type I pneumococcus	1
Land	ST+S	Type I pneumococcus	1
Rueszger and Hamburger	ST+S	Type III pneumococcus	1
Knoll	ST	β -hemolytic streptococcus	1
Dietel and Kaiser	ST	Staphylococcus aureus	1
Sadock and Nielsen	ST+NaST§	Staphylococcus aureus	1
Lyons	ST+H	Staphylococcus	2
Donovan	NaST	Staphylococcus	1
Weary and Lyons	ST	Staphylococcus	4
Gourlay and Molitor	ST	Meningococcus	1
Pulver	ST	Meningococcus	24
Banks	ST+NaST	Meningococcus	94
Laesen and Roelsen	ST	Meningococcus	6

* ST Sulfathiazole.

† S Type-specific antiserum.

‡ SMT Sulfamethylthiazole.

§ NaST Sodium salt of sulfathiazole.

|| H Heparin.

TABLE III

Pneumococcic meningitis recoveries attributed to sulfanilamide

Year	Investigator	Treatment	Number of typed recoveries	Total number recoveries
1937	Mertins and Mertins	SA*	IV	1
	Mitchell and Tracheler	SA+O†	V	1
	Basman and Perley	SA	V	1
1938	Latto	N‡	I	1
	Rowe	SA+N	V	1
	Tidder, Eck, and Grossiard	SA+S§	I	1
	Landon	N	I	1
	Hubert	III	III	1
	Finland, Brown and Rauh	SA+S	III, VII, XVII, XIX, XXVIII, XXVIII, XIV, XX, XXXI	6
	Allan, Mayer and Williams	SA	SA	3
	Young	SA	SA	1
	Gubner	SA	III	1
	Moore	SA	III	1
	Query	SA+S	VII	1
	Brasseur	SA	I	1
	Magruder and Nichols	SA	III	1
1939	Dereux	SA+S	SA	1
	Martin	SA	SA	1
	Cathala	SA	SA	1
	Kreinin	SA+S	VII	1
	SA	SA	I, IV, VI, VII, XIII, XXIX, XXXI	7
	SA	SA	SA	1
	Gray and Adams	SA+S	SA	1
	Goldman	SA	IV	1
	Canuyt	SA	IV	4
	Lisansky and Pembroke	SA	XV	1
	Toomey and Roach	SA+S	III	1
	Silverman and Thorne	SA	III	1
	Welch and Martin	SA+N	III	1
	Sappington and Favorite	SA	XXVIII	1
	Stewart and Martin	SA+N	III	1
	Hodes, Gimbell and Barnett	SA	SA	1
1940	Yampolsky	SA	I	1
	Diehl	SA	III	1
	SA+S	SA+S	III	1
	Hamby, Sherman, Greene and Whitebeck	SA	I, VI, XVII, XXXI	4
	Rhoads, Hoyne, Levin, Horswell, Reals and Fox	SA	SA	1
	Total		42	55

* SA Sulfanilamide.

† O Optochin.

‡ N Neoprontosil.

§ S Serum.

tions, fostered perhaps by the successful treatment of experimental Type I, II, and III pneumococcic meningitis in rats (7, 21, 22), has resulted in reports of 55 cases of pneumococcic meningitis whose recovery is attributed to sulfanilamide and, in several instances, to the less effective neoprontosil (Table III). Among the recoveries, 42 cases were typed and 16 received serum in conjunction with the chemotherapy. Similarly, sulfapyridine, which was considered the drug of choice for treating pneumococcic infections before the advent of sulfathiazole, is reported to have saved 88 cases of pneumococcic meningitis (Table IV). In this series, 57 cases were typed and 29 received type-specific serum as an adjuvant to the chemotherapy. A number of recoveries which received both sulfanilamide and sulfapyridine were not included in Tables III and IV.

In view of the fact that a review of the literature prior to the introduction of the sulfonamides showed but 30 recoveries from pneumococcic meningitis during the preceding 15 years (23), the report of 55 cures by sulfanilamide within the last 4 years, and four cures by sulfathiazole within less than one year leaves no reasonable doubt of the efficacy of these drugs. However, the conclusion reached in a recent review (24) that sulfanilamide and sulfapyridine were equally effective in treating pneumococcic meningitis is almost as unjustified on the basis of the statistical evidence available as the assertion that sulfanilamide and sulfathiazole are of questionable value in this disease.

Because of the lack of decisive data at present, it is not possible to judge which of the sulfonamide drugs, if any, is significantly superior in the clinical treatment of pneumococcic meningitis. Future decision on this point awaits not only the accumulation of sufficiently large numbers of cases treated with the various sulfonamides but the inclusion of the fatalities along with the cures in the data reported. Pertinent information concerning the efficacy of new drugs may also be obtained by the direct study of experimental pneumococcic meningitis and it is suggested that this method be used in preference to a reliance on the drug concentration in the cerebrospinal fluid.

CONCLUSIONS

1. Sulfanilamide, sulfapyridine, sulfathiazole, and sulfamethylthiazole are equally effective in the treatment of experimental Type II (Binda) pneumococcic meningitis in rats.

2. The sulfathiazole content of the brains of rats which received 300 mgm. of sulfathiazole orally in 24 hours averaged 1.6 mgm. per 100 grams at the time the blood averaged 5 to 6 mgm. per 100 cc.

3. Data are presented which show that clinical pneumococcic meningitis has been successfully treated with sulfanilamide as well as with sulfapyridine.

4. Other clinical data show pneumococcic, streptococcic, staphylococcic and meningococcic meningitis recoveries which were attributed to sulfathiazole therapy in spite of the generally recognized low concentration of this drug in the cerebrospinal fluid.

5. Because of the lack of decisive data at present, it is not possible to judge which of the sulfonamide drugs is significantly superior in the clinical treatment of pneumococcic meningitis. Future decision on this point awaits not only the accumulation of sufficiently large numbers of cases treated with the various sulfonamides but also the inclusion of the fatalities along with the cures in the data reported.

Note: While this paper was in press, Davis (25) presented experimental evidence which indicated that the sulfonamides are more or less closely bound to the albumen fraction of the blood, and for this reason the ratio of their "apparent" concentration in the blood to their actual concentration in the cerebrospinal fluid should not be used as a guide to the choice of drug for treating meningitis.

BIBLIOGRAPHY

1. Long, P. H., The clinical use of sulfanilamide and its derivatives in the treatment and prophylaxis of certain infections. *Bull. New York Acad. Med.*, 1940, 16, 732.
2. Long, P. H., The clinical use of sulfanilamide, sulfapyridine, sulfathiazole, sulfaguanidine, and sulfadiazine in the prophylaxis and treatment of infections. *Canad. M. A. J.*, 1941, 44, 217.
3. Alexander, H. E., Treatment of bacterial meningitis. *Bull. New York Acad. Med.*, 1941, 17, 100.
4. Bowers, W. C., Infections of the middle ear and nasal sinuses. *Bull. New York Acad. Med.*, 1941, 17, 453.
5. Bratton, A. C., and Marshall, E. K., Jr., A new coupling component for sulfanilamide determination. *J. Biol. Chem.*, 1939, 128, 537.
6. Cooper, F. B., Gross, P., and Lewis, M., Determination and distribution of sulfathiazole in blood. *Am. J. Clin. Path.*, 1942, 12, 149.
7. Gross, P., and Cooper, F. B., The chemotherapy of experimental Type II pneumococcic meningitis. *Am. J. M. Sc.*, 1939, 197, 609.
8. Gross, P., Cooper, F. B., and Lewis, M., Repair in experimental pneumococcic meningitis. *Am. J. Path.*, 1939, 15, 193.
9. Gross, P., Cooper, F. B., and Lewis, M., Urinary calculi caused by sulfapyridine. *Urol. and Cutan. Rev.*, 1939, 43, 299.
10. Gross, P., Cooper, F. B., and Lewis, M., The fate of urinary calculi caused by the administration of sulfapyridine. *Urol. and Cutan. Rev.*, 1939, 43, 439.
11. Gross, P., Cooper, F. B., and Scott, R. E., Urolithiasis medicamentosa. *Urol. and Cutan. Rev.*, 1940, 44, 205.
12. Buttle, G. A. H., Gray, W. H., and Stephensen, D., Protection of mice against streptococcal and other

- infections by *p*-aminobenzenesulphonamide and related substances. *Lancet*, 1936, 1, 1286.
13. Whitby, L. E. H., The assessment of the efficiency of chemotherapeutic substances. *Practitioner*, 1937, 139, 650.
 14. Pneumonia Commission, Medical Society of State of Pennsylvania, Pneumonia. *Weekly Roster and Med. Digest*, 1938, 33, 647, 686.
 15. Current Comment, Sulfanilamide-pyridine. *J. A. M. A.*, 1938, 111, 2122.
 16. Long, P. H., Bliss, E. A., and Feinstone, W. H., The effects of sulfa-pyridine, sulfanilamide and related compounds in bacterial infections. *Pennsylvania M. J.*, 1939, 42, 483.
 17. Whitby, L. E. H., The treatment of septicemia. *Current Med. Digest*, 1939, 6, 321.
 18. Buttle, G. A. H., Sulphanilamide chemotherapy. *Lancet*, 1939, 2, 371.
 19. Buttle, G. A. H., Pharmacology of the sulphanilamide group of drugs. *Brit. M. J.*, 1939, 2, 269.
 20. Council on Pharmacy and Chemistry, Sulfanilamide. *J. A. M. A.*, 1939, 112, 733.
 21. Gross, P., Cooper, F. B., and Lewis M., Therapeusis of Type I pneumococcic meningitis in rats. *Am. J. M. Sc.*, 1939, 198, 66.
 22. Cooper, F. B., Gross, P., and Lewis, M., Sulfapyridine, sulfanilamide and specific antiserum in experimental Type III pneumococcic infections. *J. Clin. Invest.*, 1939, 18, 423.
 23. Mertins, P. S., and Mertins, P. S., Jr., Meningitis due to the Type IV pneumococcus, with recovery. *Arch. Otolaryng.*, 1937, 25, 657.
 24. Steele, C. W., and Gottlieb, J., Treatment of pneumococcic meningitis with sulfanilamide and sulfapyridine. *Arch. Int. Med.*, 1941, 68, 211.
 25. Davis, B. D., Binding of sulfonamides by plasma proteins. *Science*, 1942, 95, 78.

COMPARISON OF RESULTS OF THE NORMAL BALLISTOCARDIOGRAM AND A DIRECT FICK METHOD IN MEASURING THE CARDIAC OUTPUT IN MAN¹

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Starr and his associates (1) have demonstrated several characteristics of the ballistocardiographic method of cardiac output determination which recommend it for clinical use. The technique is simple, the apparatus is highly sensitive to small variations in stroke volume, the results in the same individual are consistent, and the limits of normality are well defined. The usefulness of the method for investigative work, however, depends in addition upon its degree of accuracy.

Preliminary information as to the accuracy of the method has been provided by Starr *et al.* by comparing results calculated from the ballistocardiogram with those obtained by other methods of cardiac output determination. Almost simultaneous cardiac output determinations by both the ethyl iodide method and the ballistocardiograph were performed on 30 subjects (2). Of these, the 25 best cases showed values of cardiac output averaging 3.9 per cent smaller by the ballistocardiograph than by the ethyl iodide method.

A larger discrepancy was found between a group of 106 normal subjects studied by the ballistocardiograph (1) and two other groups of normal subjects studied by the ethyl iodide (3) and acetylene methods (4). Values for cardiac output by the ballistocardiograph averaged 14.4 per cent smaller than by the ethyl iodide method and 13.6 per cent smaller than by the acetylene method. It must be noted in addition that standard basal conditions were not obtained in the group studied with the ballistocardiograph. If more rigid basal conditions had prevailed as in the two series studied by the foreign gas methods, it is fair to assume that the figures for cardiac output by the ballistocardiograph would have been somewhat lower, thus increasing the discrepancy. The comparisons presented to date do not satis-

factorily evaluate the accuracy of the ballistocardiographic method and suggest the need for further data.

The present study is a comparison of cardiac output determined by a method based on the Fick principle and by the ballistocardiograph.

METHOD

The technique of cardiac output determination based on the Fick principle involved collection of mixed venous blood, arterial blood, and expired air. Mixed venous blood was withdrawn from the right auricle by means of a catheter. The method of catheterization has been described by two of the present authors (5). It was modified in individuals whose arm veins were not large enough to permit the introduction of a 10 gauge needle. In these cases the vein was exposed and the catheter introduced directly through a small slit in the vein. In order to insure that the gas concentration of the mixed venous blood remained unaltered, the samples were collected over mercury instead of under oil. The mercury sampling tube was previously prepared by introducing the anticoagulant, potassium oxalate and sodium fluoride, and allowing it to dry. Any gas trapped in the tube was removed by evacuating and expelling. Thus prepared, the tube was attached to the catheter. After interrupting the previous flow of saline, 3 cc. of blood were withdrawn through a side arm to wash out the saline, and 10 to 15 cc. of blood then drawn into the sampling tube by slight suction created by lowering the levelling bulb. This procedure required about 20 to 30 seconds. The blood was immediately mixed with the anticoagulant by inverting the sampling tube several times. Pipettes were filled directly for analysis, and any remaining blood was kept on ice under a slight positive pressure. A sample of arterial blood was taken under oil from the femoral artery, starting 15 seconds after the sampling of the mixed venous blood had begun. The expired air was collected in a Tissot spirometer, beginning 30 seconds before the mixed venous blood sampling and continuing for 1½ minutes. The expired air and blood samples were analyzed in duplicate for carbon dioxide and oxygen content.

The technique of obtaining mixed venous blood through a catheter in the right auricle made possible two determinations of cardiac output from each set of samples.

¹ Under a grant from the Commonwealth Fund.

The two values obtained from

$$\frac{\text{Total CO}_2 \text{ output in cc. per minute}}{\text{CO}_2 \text{ arteriovenous difference, cc. per liter of blood}}$$

and from

$$\frac{\text{Total O}_2 \text{ intake, in cc. per minute}}{\text{O}_2 \text{ arteriovenous difference, cc. per liter of blood}}$$

acted as independent checks upon each other.

Ballistocardiographic tracings were taken immediately before and immediately after each direct Fick determination.² Estimates of cardiac output from ballistocardiograms were made by two independent persons. Complexes covering at least one respiratory cycle were chosen. If the influence of respiration upon the tracing varied, complexes covering several cycles were calculated. The wave area formula was used (Formula 16 in Starr's original paper (2)). Triangles were constructed by extending the sides of waves I and J and the areas under the waves were approximated by calculating the areas of the triangles. The altitude was measured with a divider to the half millimeter. The base of the triangle was measured from the timer lines on the tracing, the reading being accurate to within 0.04 seconds and estimated to within 0.02 seconds. At the end of each experiment the bed was calibrated and the formula corrected accordingly.

RESULTS

Thirty-one attempts in 19 different subjects were made to introduce the catheter into the right auricle. Twenty-four attempts in 18 subjects were successful. Forty-eight direct Fick determinations were attempted in these 18 subjects. Twenty-one determinations in 13 subjects were satisfactory. In 5 of these 21 instances, the ballistocardiograms were discarded, either because the difference in pulse rate between determinations was too large (more than 3 beats), or because of technical error in taking the tracing. Thus in 16 instances, involving 12 patients, both direct Fick determinations and the ballistocardiograms were technically satisfactory.

All data from which cardiac output determinations by the direct Fick method were calculated are tabulated in Table I. The subjects were not under standard basal metabolic conditions, and several of them were not normal. The values for cardiac output can therefore only be used for comparison with those obtained by another method

employed almost simultaneously and under identical circumstances. It is of interest, however, that, after excluding subjects who had received angiotonin or had been subjected to a prolonged surgical operation, the average oxygen arteriovenous difference per 100 cc. of blood was 4.8 cc. with extremes of 4.0 cc. and 6.0 cc. This is somewhat lower than average figures obtained with the foreign gas methods in normal subjects under standard basal conditions but is well within the range of variation.

The direct Fick method as used here provides a check within itself. The respiratory quotient derived in the usual way by analysis of expired air can be checked against a respiratory quotient derived from blood gas analysis by the ratio

$$\frac{\text{CO}_2 \text{ arteriovenous difference (volume per cent)}}{\text{O}_2 \text{ arteriovenous difference (volume per cent)}}$$

These values are shown in Columns 4 and 11. In only 4 determinations is the difference 0.04 or greater, being 0.04 in 2 cases, 0.06 in 1 case, and 0.07 in another.

The two figures for cardiac output, one derived from the carbon dioxide data, the other from the oxygen data, are listed in Columns 12 and 13. In only 2 instances was the difference greater than 0.24 liters per minute, being 0.35 and 0.43 liters per minute in these cases. On an average, each determination differed by only 4.3 per cent from the mean value of the pair.

In Table II are tabulated data comparing stroke volumes determined by the direct Fick method and calculated from the ballistocardiographic tracings. The figures for the direct Fick determinations were calculated by averaging the values for cardiac output per minute calculated separately from the carbon dioxide and the oxygen data, and dividing by pulse rate; the figures for the ballistocardiographic determinations were obtained by averaging the results before and after the direct Fick. The ballistocardiograms of the first 2 cases, while apparently technically correct, showed complexes which were small and which in 1 case were irregular. Since the error in calculating cardiac output from these tracings may have been large, the cases have not been included in the final comparison of the two methods. In the remaining 14 experiments, the stroke volume by direct Fick

² The ballistocardiograph used was designed by Drs. Dugold E. S. Brown and Homer A. Smith on the same principle as Dr. Starr's apparatus (see appendix).

TABLE I

Data obtained in 21 determinations of cardiac output by the direct Fick method

Subject	1		2	3	4	5	6	7	8	9	10	11	12	13
	Date		Lung gas exchange			Blood gas exchange							Cardiac output	
			CO ₂ output, cc. per minute	O ₂ intake, cc. per minute	Respiratory quotient	CO ₂ con- tent		CO ₂ difference, volume per cent	O ₂ con- tent		O ₂ difference, volume per cent	Ratio of CO ₂ to O ₂ difference	from CO ₂ data, liters per minute	from O ₂ data, liters per minute
						M. V. B. volume per cent	A. B. volume per cent		A. B. volume per cent	M. V. B. volume per cent				
G.B. Essential hypertension, cardiac failure	October	31, 1940	221	272	0.813	53.9	47.2	6.7	12.4	4.1	8.3	0.81	3.30	3.28
P.T. Carcinoma of liver	November	27, 1940	193.5	282	0.687	50.7	47.0	3.7	12.1	6.8	5.3	0.70	5.23	5.32
F.L. Carcinoma of cardia	December	31, 1940	192	225	0.854	54.7	51.2	3.5	14.0	10.0	4.0	0.88	5.49	5.63
P.H. Normal	February	18, 1941	183	235	0.778	55.0	50.1	4.9	19.1	13.1	6.0	0.82	3.74	3.92
F.K. Normal	February	20, 1941	252	273	0.922	61.5	57.2	4.3	17.8	13.3	4.5	0.95	5.86	6.07
	February	27, 1941	246	262	0.942	57.4	53.6	3.8	16.4	12.4	4.0	0.95	6.48	6.55
	Same*		243	277	0.878	58.4	53.1	4.5	15.7	10.4	5.3	0.85	5.40	5.23
W.O'Br. Normal	March	4, 1941	221	263	0.842	52.8	48.8	4.0	17.9	13.0	4.9	0.82	5.53	5.33
L.K. Carcinoma of bronchus	March	12, 1941	223	248	0.899	50.5	45.7	4.8	15.7	10.5	5.2	0.92	4.65	4.78
H.W. Normal	Same		221	251	0.881	50.5	45.7	4.8	15.7	10.4	5.3	0.91	4.61	4.74
W.O'Bo. Normal	March	25, 1941	223	240	0.929	54.1	50.1	4.0	16.3	11.8	4.5	0.89	5.58	5.34
M.M. Chronic pulmonary tuberculosis	April	2, 1941	243	296	0.821	52.6	48.0	4.6	17.2	11.7	5.5	0.84	5.29	5.37
J.L. Normal	Same		208	259	0.802	51.5	47.2	4.3	17.2	11.9	5.3	0.81	4.84	4.89
J.B. Normal	May	8, 1941	139.5	195.5	0.794	53.2	49.8	3.4	14.6	10.4	4.2	0.81	4.10	4.17
J.D. Chronic pulmonary tuberculosis	Same§		142.5	190.5	0.747	51.2	47.0	4.2	13.7	8.5	5.2	0.81	3.40	3.75
J.B. Normal	May	16, 1941	244	311	0.786	54.9	51.3	3.6	17.7	13.0	4.7	0.77	6.76	6.69
J.D. Normal	Same†		240	314	0.765	54.2	50.4	3.8	16.8	12.0	4.8	0.79	6.31	6.54
J.B. Normal	June	4, 1941	199	242	0.822	51.3	47.6	3.7	19.0	14.3	4.7	0.79	5.38	5.27
J.D. Chronic pulmonary tuberculosis	Same‡		216	262	0.824	51.4	46.7	4.7	19.9	14.3	5.6	0.84	4.60	4.68
	June	18, 1941	181	234	0.774	49.9	46.2	3.7	18.4	14.0	4.4	0.84	4.89	5.32
	Same§		211	265	0.796	45.6	41.7	3.9	16.9	12.1	4.8	0.81	5.41	5.52

* Four minutes after injection of angiotonin.

† Thirty seconds after injection of angiotonin.

‡ Three minutes after injection of angiotonin.

§ At the end of prolonged surgical operation.

averaged 18.5 per cent larger than by ballistocardiograph, with extremes of +10.6 per cent and +33.5 per cent. This difference between measurements by the two methods is significant for $P=0.05$.

On subjects F. K. and J. B., cardiac output determinations were made before and after the injection of 1 cc. of angiotonin. The percentage difference between the values for stroke volume obtained simultaneously by the two methods remained remarkably constant whether or not there were changes in the absolute values of stroke volume due to the action of the drug.

The results of the 16 almost simultaneous pairs of cardiac output determinations have been plotted in Figure 1. The values corresponding to the

first 2 subjects in Table II are identified by the open circles. The other 14 points are all above the line of identity. Their distribution is indicative of a very high correlation and a systematic deviation. It can furthermore be inferred that both methods are consistent within themselves. Because of the smallness of the sample, the drawing of a regression line and the calculation of its equation are not justified.

DISCUSSION

The attempt to compare almost simultaneous cardiac output determinations by the direct Fick method and by the ballistocardiograph resulted in a high proportion of failures for a variety of reasons. Among the successful cases in whom a

TABLE II

Sixteen simultaneous determinations of stroke volume by the direct Fick method and by the ballistocardiograph in 12 subjects

Subject	Pulse	Stroke volume		Difference (Direct Fick)-(Ballistocardiograph)	Ballistocardiograph record
		Direct Fick*	Ballistocardiograph†		
G.B.....	93	cc. 35.4	cc. 38.6	cc. -3.2	Complexes irregular, small
L.K.....	108	43.5	59.6	-16.1	Small complexes
P.T.....	112	47.2	41.0	+6.2	Good
R.L.....	68	81.2	73.1	+8.1	Good
P.H.....	56	68.4	61.6	+6.8	Good
P.K.....	61	97.8	84.8	+13.0	Good
	74‡	88.1	73.5	+14.6	Good
	68§	78.1	64.8	+13.3	Good
W.O'Br.....	80	68.0	57.1	+10.9	Good
H.W.....	75	72.8	63.4	+9.4	Good
W.O'Bo.....	64	83.1	73.1	+10.0	Good
	65	75.0	67.8	+7.2	Good
L.....	96	69.7	52.2	+17.5	Good
B.....	65‡	81.1	62.2	+18.9	Good
	58§	80.7	64.0	+16.7	Good
D.....	84	60.8	49.0	+11.8	Good
Average.....	77.6	70.7	61.6	+9.1	
Average of 14 with good ballistocardiograph records.....	73.3	75.1	63.4	+11.7	

* Stroke volume obtained by averaging figures in two last columns of Table I and dividing by mean pulse rate.

† Stroke volume obtained by averaging figures of 2 ballistocardiograms taken immediately before and immediately after the direct Fick determination.

‡ Before the injection and § after the injection of angiotonin.

comparison seemed justified, there was a systematic deviation in the results. The following analysis of some of the technical and theoretical considerations involved in the two methods may help to explain these findings.

The direct Fick determination required first the placing of the catheter in the right auricle. In 24, or 77 per cent, of 31 attempts this procedure was successful. Failures resulted from inability to introduce the catheter into the basilic vein, from obstruction to its course as it was threaded along, or from inability to make it follow the desired venous channels. The apparent harmlessness of the procedure was confirmed, occasional thrombosis of the basilic vein being the only untoward reaction. In more than 50 attempts

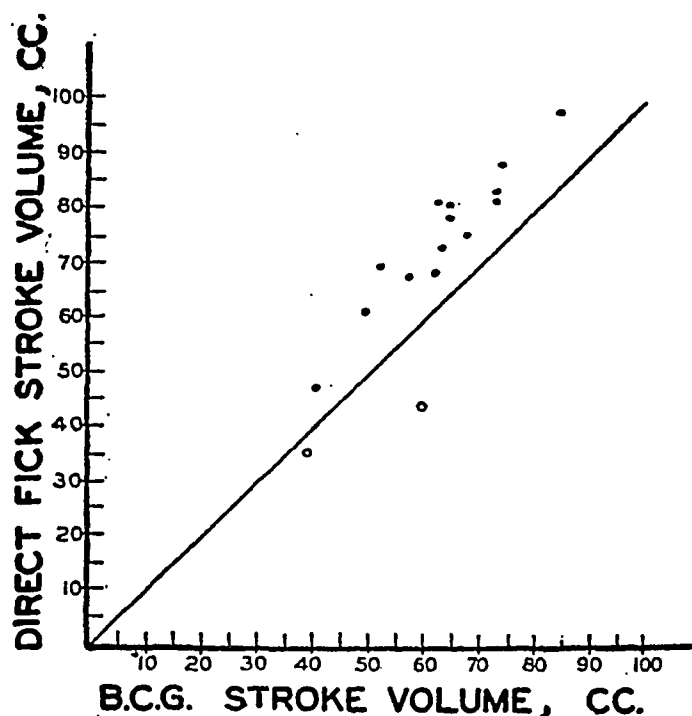


FIG. 1. SIXTEEN INDIVIDUAL MEASUREMENTS OF STROKE VOLUME OBTAINED BY THE DIRECT FICK METHOD PLOTTED AGAINST THE SAME MEASUREMENTS CALCULATED FROM SIMULTANEOUS BALLISTOCARDIOGRAPHS

Open circles represent measurements in 2 subjects with rapid pulse, small and/or irregular ballistic complexes. Straight line is line of identity (see text).

including both those here reported and others to be reported elsewhere, the only physiological responses were a small rise in ventilation and a small degree of sinus bradycardia. Studies now in progress on surgical shock have shown that with the continuous flow of saline maintained at a rate of about 15 drops per minute, the catheter can be kept in position for as long as 3 hours with no inconvenience to the patient.

In the Fick determinations the chief difficulty arose in obtaining simultaneously, and analyzing in duplicate, samples of mixed venous blood, arterial blood, and expired air. In 21, or 44 per cent, of 48 attempts the determinations were satisfactory. The collection of expired air presented no special difficulties. The sampling of arterial blood, following infiltration of the femoral artery with novocaine, was in most cases technically easy and apparently painless. Stimuli arising from the arterial puncture may on occasion have caused momentary increase in the cardiac output, but it is doubtful if this occurred in the selected group of cases in which the pulse rate varied by no more than 3 beats. The sampling of venous

blood from the right auricle was somewhat complicated by the necessity for washing the catheter free of saline and for taking the strictest precautions against contamination with air. There is also the possibility that samples taken from the right auricle may not always have been representative of thoroughly mixed venous blood. While this possibility has yet to be thoroughly investigated, it is doubtful that it was a source of error in the selected group in which the respiratory quotient obtained from the arteriovenous differences checked reasonably well with the respiratory quotient obtained from analysis of expired air. Certainly the high proportion of failures in performing the direct Fick determination was due mainly to technical difficulties, since error at any point in manipulating the large number of samples required that the entire experiment be discarded.

The determination of cardiac output by the direct Fick method, although difficult, is based on fundamental physiological principles and has checks within itself. The degree of accuracy is therefore thought to be high. Any discrepancy between the results obtained by this method and by the ballistocardiograph may be assumed to be due to error in the latter.

The ballistocardiograms were satisfactory in 16, or 76 per cent, of the 21 cases in which the Fick determinations were satisfactory. The taking of these tracings was an extremely simple matter provided the optical recording and timing devices functioned properly. The patient was required simply to remain absolutely quiet with his feet firmly against the foot board. Only tracings which were normal in shape were used, since we were interested exclusively in the normal ballistocardiogram. In the comparison of methods several ballistocardiograms taken before and after the direct Fick were not acceptable because the pulse rate varied by more than 3 beats.

Consideration of all possible sources of error related to the ballistocardiograph would require an evaluation of the fundamental assumptions upon which Starr's formulae were based. Assuming, however, for lack of better knowledge, that every other entry from which the formulae were derived is correct, there remains one factor which is open to doubt,—namely, the estimation of the internal cross-section of the aorta. The standard tables, relating aortic cross-section to age and body surface, were compiled by Bazett from data collected by Suter on autopsy material (6). The magnitude of the error introduced by applying

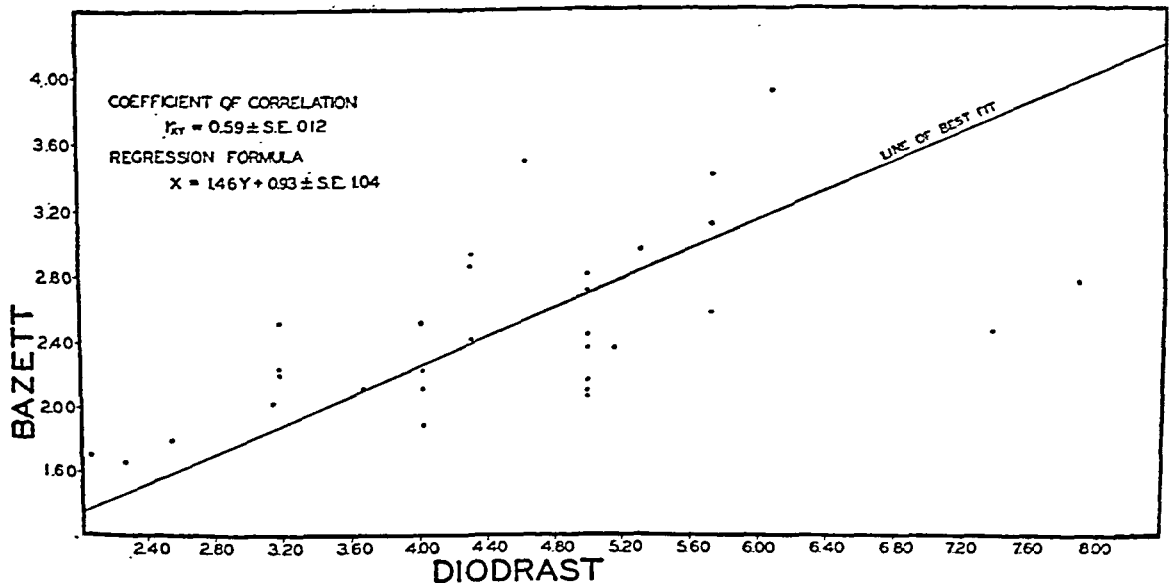


FIG. 2. INTERNAL CROSS-SECTION AREA OF AORTA IN SQ. CM. IN 31 NORMAL SUBJECTS

Estimates of this measurement obtained from Bazett's table are compared with measurements calculated from the smallest diameter of the aorta near the aortic ring after diodrast visualization.

these figures to living subjects is not known, but it can readily be shown that any considerable error in the estimate of aortic cross-section will significantly affect the calculated cardiac output. For example, a 50 per cent error in aortic cross-section (let us say, 3 sq. cm. instead of 2 sq. cm.) will result in a 22 per cent error in stroke volume as calculated by Starr's wave area formula. The error in stroke volume varies with the error in aortic cross-section according to the approximate formula $\frac{dS}{S} = \frac{dA}{2A}$, where S = stroke volume and A = aortic cross-section.

To test the accuracy of Bazett's figures, the internal diameter of the ascending aorta at its narrowest point was measured directly in 31 normal subjects after visualization of the aorta with diodrast.³

The correlation found by Bazett between age, body surface, and aortic cross-section was confirmed, but a more rapid increase in aortic cross-section with increasing body surface was found in each age group. Because of this the diodrast measurements are in general considerably higher than the estimates from Bazett's tables. The values obtained by direct visualization are plotted against estimates obtained from Bazett's data in Figure 3. The correlation coefficient $r_{xy} = 0.53$, $S.E. \mp 0.12$ is significant, for P = less than 0.01. To apply a correction factor to Bazett's figures by using the calculated regression formula is unjustified because of the small size of the sample, the lack of information regarding variations in the size of the aorta on successive diodrast visualizations, and the large error involved in the use of this formula as shown by the standard error.

Since a correction factor could not be applied, the diameter of the aorta was measured by diodrast visualization in 5 of the subjects in whom the cardiac output had been measured by both direct Fick and ballistocardiographic methods. These 5 subjects were representative of the larger group since the average difference between Fick and ballistocardiographic determinations was very nearly the same as the average difference for the entire group of 14, being 17.3 per cent as opposed to 18.5 per cent. It is significant, there-

³ The aortic visualization had been performed by Drs. Robb, Steinberg, and Roche, who very kindly placed this material at our disposal.

TABLE III

Comparison between direct Fick and ballistocardiograph stroke volume in 5 subjects. Influence of direct measurement of the aorta (diodrast) in the determination of stroke volume by ballistocardiograph

Subject	Age	Body surface area	Aortic cross section area		Stroke volume		
					Ballisto-cardiograph		Direct Fick
			Bazett	Dio-drast	Bazett	Dio-drast	
		sq. m.	sq. cm.		cc.		
W.O'Bo...	49	1.88	4.08	5.72	70.2	83.2	79.1
P.H.....	52	1.80	4.02	5.31	61.6	70.7	68.4
J.L.....	39	1.68	3.02	5.73	52.2	71.5	69.7
J.D.....	21	1.79	2.38*	4.15	49.0	64.3	60.8
F.L.....	61	1.66	4.70	5.88	73.1	81.8	81.2

* Estimated on basis of best weight.

fore, that, as shown in Table III, the difference between direct Fick and ballistocardiographic results averaged only 3.5 per cent when the ballistocardiograms were re-calculated using diodrast measurements of the aortas.

It thus appears probable that the use of Bazett's figures for the internal cross-section of the aorta introduces an error into the calculation of cardiac output by Starr's wave area formula, and that in the 5 cases studied, correction of this error by direct measurement of the aortas resulted in good agreement between ballistocardiographic and direct Fick methods.

SUMMARY AND CONCLUSIONS

1. The accuracy of the ballistocardiographic method of cardiac output determination was tested by comparing it with a method based on the Fick principle.

2. The technique of the direct Fick determination, involving catheterization of the right auricle, was discussed.

3. Fourteen almost simultaneous pairs of cardiac output determinations were compared, in which the following criteria were satisfied: pulse rate varied less than 4 beats; ballistocardiograms were normal in shape, regular, and easily readable; cardiac output calculated separately from oxygen consumption and from carbon dioxide elimination checked closely.

4. Cardiac output as determined by the direct Fick method was found to be larger by 18.5 per cent than the value calculated from the ballisto-

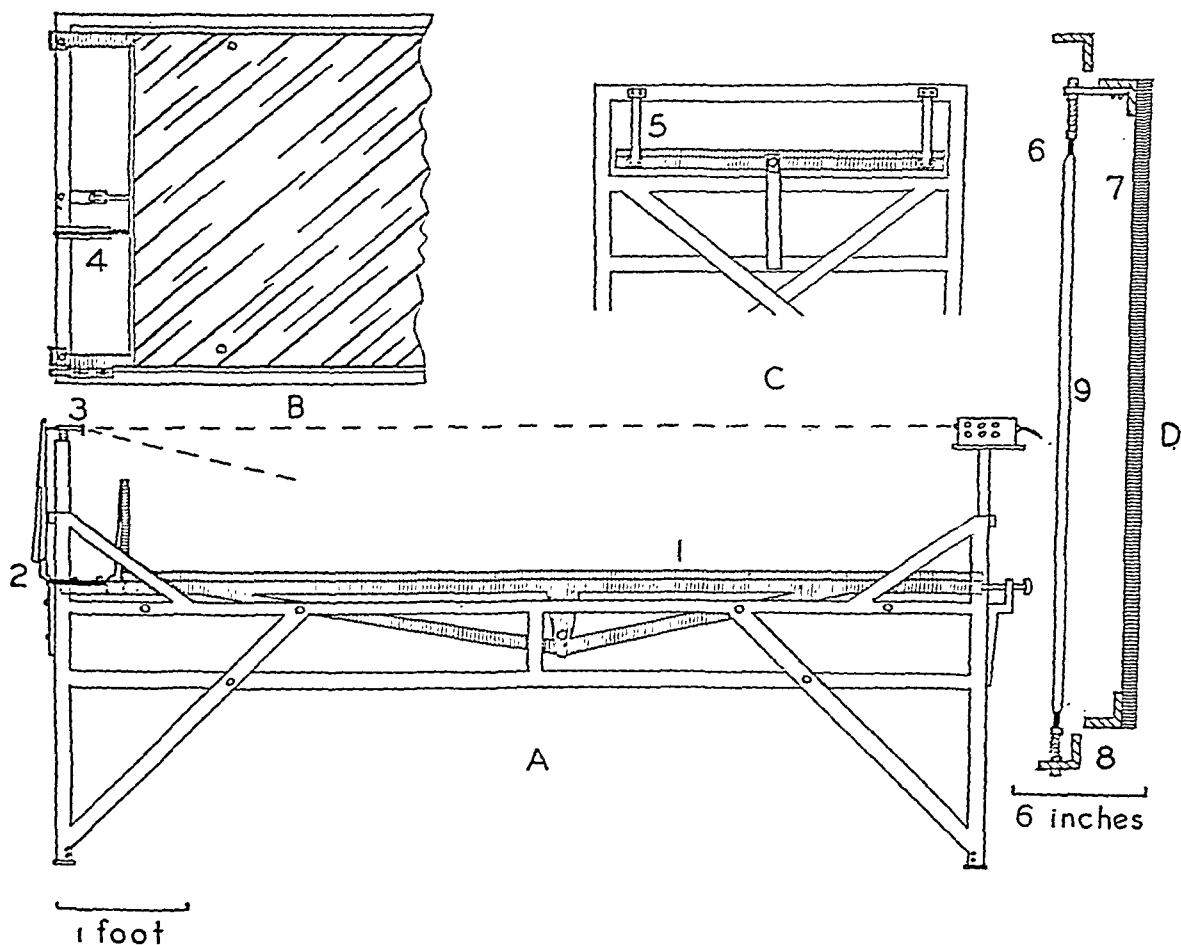


FIG. 3.

- A. Side view of the ballistograph showing platform (1), driving lever (2), recording lever (3).
 B. Top view, showing position of platform with respect to frame and position of calibrating spring (4).
 C. End view, showing platform suspended by spring steel strips (5).
 D. Cross-section of platform and frame, showing arrangement of strut (9) between frame (8) and platform (7), thereby preventing lateral movement. At either end, the $\frac{3}{8}$ inch strut is connected to platform and frame, respectively, by a strip of spring steel $\frac{1}{2}$ inch by $\frac{1}{32}$ inch.

cardiogram, using Bazett's tables for the internal cross-section of the aorta.

5. Using figures for aortic cross-section obtained by diodrast visualization in 5 cases, cardiac output as calculated from the ballistocardiogram was found to check very closely with the values obtained by the direct Fick method, the average difference being 3.5 per cent.

6. On the basis of these findings, it is suggested that the accuracy of cardiac output determination with the ballistocardiograph may be improved (1) by correcting the calculated value by an amount equal to the average error found experimentally, *i.e.* 18.5 per cent, or (2) by introducing in the formula a value for internal cross-section of the aorta based on diodrast visualization.

APPENDIX

THE DESIGN OF THE BALLISTOCARDIOGRAPH

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The design of the ballistocardiograph follows, in general, the principles of that of Isaac Starr (2). The major problem faced in a portable model is the elimination of distortion and vibrations arising from faulty design. The short suspension elements and the position of the driving lever and optical recording units were selected as being most suitable for the purpose. Damping of the platform would greatly improve the records but does not appear necessary at the present time.

The framework throughout is of angle iron, $1\frac{1}{4}$ inches wide and $\frac{1}{8}$ inch thick, reinforced with bands $1\frac{1}{4}$ inches by $\frac{1}{8}$ inch. The platform is covered with $\frac{7}{8}$ inch plywood and, if desired, may be covered with a sponge rubber mat 1 inch in thickness.

The platform is suspended by four strips of spring steel, 5 inches long, $\frac{5}{8}$ inch wide, and $\frac{1}{32}$ inch thick. It is fitted with a locking device at the foot to immobilize the platform when necessary. Lateral stabilization of the platform is obtained by struts (Figure 1, D, 6) of the type employed by Starr.

The driving lever (Figure 1, A, 2) is a strip of tool steel, $5\frac{1}{2}$ inches by $\frac{3}{4}$ and $\frac{1}{4}$ inch in thickness. The platform rests against the lever $\frac{1}{2}$ inch from the fulcrum through a knife-edge strip of tool steel. An aluminum extension, braced to eliminate secondary vibrations, provides a light extension of the driving lever to its attachment to the recording lever (Figure 1, A, 3).

The recording lever, of the torsion wire type, provides a positive connection to the driving lever at all times through a light piece of chain. The mirror is mounted rigidly on the torsion band to eliminate secondary free vibrations. It is front surfaced with aluminum and has a focal length of 0.25 D.

The lamp, mounted on the head of the bed, has a 75 watt projection bulb as a light source, a short focus condensing lens and an adjustable slit. With a 0.25 D mirror, the image of the slit comes to a focus 0.5 meters behind the lamp.

For purposes of calibration, a spring is provided which is attached between the frame and the platform at 4 (Figure 1, B). Extension of the spring two centimeters exerts a force of 1000 grams upon the platform.

The period of the platform under a given load depends on the length of the suspension strips, the elasticity of the driving lever, and the elasticity of the recording lever. If a shorter period (greater frequency) is desired, the simplest procedure is to increase the thickness of the driving lever. Some amplitude will be lost, but this can easily be corrected for by moving the camera further from the mirror. In use, the bed must rest directly on a solid floor if the free period of 15 per second is to be retained. Best results are obtained when the bed is placed close to an outside wall at right angles to the axis of vibration of the floor, if such exists.

BIBLIOGRAPHY

1. Starr, I., and Schroeder, H. A., Ballistocardiogram. II. Normal standards, abnormalities commonly found in disease of the heart and circulation, and their significance. *J. Clin. Invest.*, 1940; 19, 437.
2. Starr, I., Rawson, A. J., Schroeder, H. A., and Joseph, N. R., Studies on the estimation of cardiac output in man, and of abnormalities in cardiac function, from the heart's recoil and the blood's impacts; the ballistocardiogram. *Am. J. Physiol.*, 1939, 127, 1.
3. Starr, I., Gamble, C. J., Marjories, A., Donal, J. S., Jr., and Eagle, E., A clinical study of the action of ten commonly used drugs on cardiac output, work and size; on respiration, on metabolic rate, and on the electrocardiogram. *J. Clin. Invest.*, 1937, 16, 799.
4. Grollman, A., The Cardiac Output of Man in Health and Disease. Monograph. Charles C. Thomas, Baltimore, 1932.
5. Cournand, A., and Ranges, H. A., Catheterization of the right auricle in man. *Proc. Soc. Exper. Biol. and Med.*, 1941, 46, 462.
6. Bazett, H. C., Cotton, F. S., Laplace, L. B., and Scott, J. C., The calculation of cardiac output and effective peripheral resistance from blood pressure measurements, with an appendix on the size of the aorta in man. *Am. J. Physiol.*, 1935, 113, 312.

THE *IN VITRO* AND *IN VIVO* EFFECT OF SULFONAMIDES UPON THE STREPTOCOCCAL ANTIFIBRINOLYSIN TEST

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The streptococcal antifibrinolysin test of Tillett and Garner (1) was at first thought to be diagnostic of recent hemolytic streptococcal infections. Subsequent studies have shown, however, that the test may be non-specific, increased amounts of antifibrinolysin having been noted in other diseases, such as gonococcal arthritis (2) streptococcus viridans bacteremia (3) and pneumococcal pneumonia (4).

The present communication deals with the relation of sulfonamides to the production of antifibrinolysin. An increased resistance to fibrinolysis was found by Hines, Hoover, and Graff (5) in patients receiving sulfanilamide. This increased liquefaction time paralleled, in general, the rise or fall of the concentration of sulfanilamide in the blood stream, and the fibrinolysin time returned to its normal level in from two to five days after administration of the drug had been discontinued. From these results it seemed evident that sulfanilamide in some manner increases the antifibrinolytic properties of the tissues of the host, and it was suggested that this action may aid the bacteriostatic properties of the drug by preventing the spread of organisms throughout the body.

Since sulfonamides are used so widely at the present time, especially in diseases in which the antifibrinolysin test is of the greatest interest, it seemed worth while to attempt to confirm these results.

PROCEDURE AND RESULTS

Three strains of hemolytic streptococci were used as sources of fibrinolysin:

Number 1, a solid lytic substance, prepared from a Lancefield group A organism isolated from a human infection. The method was that of Garner and Tillett (6), and fresh solutions, containing 5 mgm. per 0.5 cc. were prepared shortly before performing each test.

Number 2, another Lancefield group A strain, selected especially because it produced considerably less fibrinolysin than the one used in making the solid substance. This was transferred daily in veal infusion broth.

Number 3, a Lancefield group C strain, isolated from the blood stream of a bacteremic patient, which was also transferred daily in veal infusion broth.

Numbers 1 and 3 regularly lysed normal human fibrin clots in twenty minutes or less. With *Number 2*, lysis usually occurred in two to four hours. The fibrin clots of patients with recent hemolytic streptococcal infections showed partial or complete resistance to liquefaction with all three lytic strains.

The antifibrinolysin tests were performed according to the method of Tillett and Garner (1), except that the plasma fibrinolysin solution was incubated for fifteen minutes before adding CaCl_2 .

Experiment 1

Studies were made of the *in vitro* effect of sulfanilamide. Antifibrinolysin tests were performed in duplicate on the plasmas of seven normal individuals. One tube contained no sulfanilamide, while the other contained the drug in a concentration of 20 mgm. per cent. Only the solid lytic substance was used in this experiment.

Results. The liquefaction times varied from five to twenty-five minutes. The time required for lysis in the tubes containing sulfanilamide was in every instance almost identical with that in the tubes to which no drug had been added. These results are in agreement with those of Huntington (7), who also found that the addition of sulfanilamide *in vitro* produces no effect upon the antifibrinolysin test.

Experiment 2

Antifibrinolysin tests were performed on six children with scarlet fever who were treated with full therapeutic doses of sulfanilamide or sulfathiazole. The tests were performed once or twice in the four or five days during which the drugs were administered. Again, only the solid lytic substance described above was used for the tests.

These patients were followed for several weeks after the drugs had been discontinued, antifibrinolysin tests being performed at weekly intervals.

Results. The liquefaction times during the period of sulfonamide administration were under twenty minutes in all instances. These determinations were made during the first five or six days of the disease, before an increase had occurred in the antifibrinolysin titer due to the streptococcal infection *per se*. Such an increase in the amount of antifibrinolysin did develop later in four of the six children, beginning during the latter part of the second week of the disease. In two, lysis occurred after several hours, while in the other two there was complete resistance to lysis at the end of twenty-four hours. Thus, it is evident that sulfonamide administration caused no increase in antifibrinolysin in these individuals who later developed high titers as a result of hemolytic streptococcal infection.

Experiment 3

Six patients on the medical wards, mostly diagnostic problems with no demonstrable organic disease, were selected. All had normal temperatures and leukocyte counts. Antifibrinolysin tests were performed on two successive days before the administration of sulfonamides in order to establish a constant base line. The three lytic substances previously described were used in all the tests. The patients were then given sulfonamides orally, an initial dose of 4 grams followed by 1 gram every four hours. Blood was collected at twenty-four hour intervals after the initial dose for antifibrinolysin tests and for determinations of sulfonamide blood levels which were made according to the method of Bratton and Marshall (8). Of the six patients, two received sulfanilamide, two sulfathiazole, and two sulfadiazine. In all instances the antifibrinolysin tests were begun within four hours after the plasma was collected.

Results. The accompanying figure shows the results in all six patients. It can be readily seen that administration of the sulfonamides had no effect whatsoever upon the results of the antifibrinolysin tests. Blood levels of the magnitude desirable for therapy of severe infections were attained in most instances. Patient 6 received sulfadiazine for two weeks, during which the

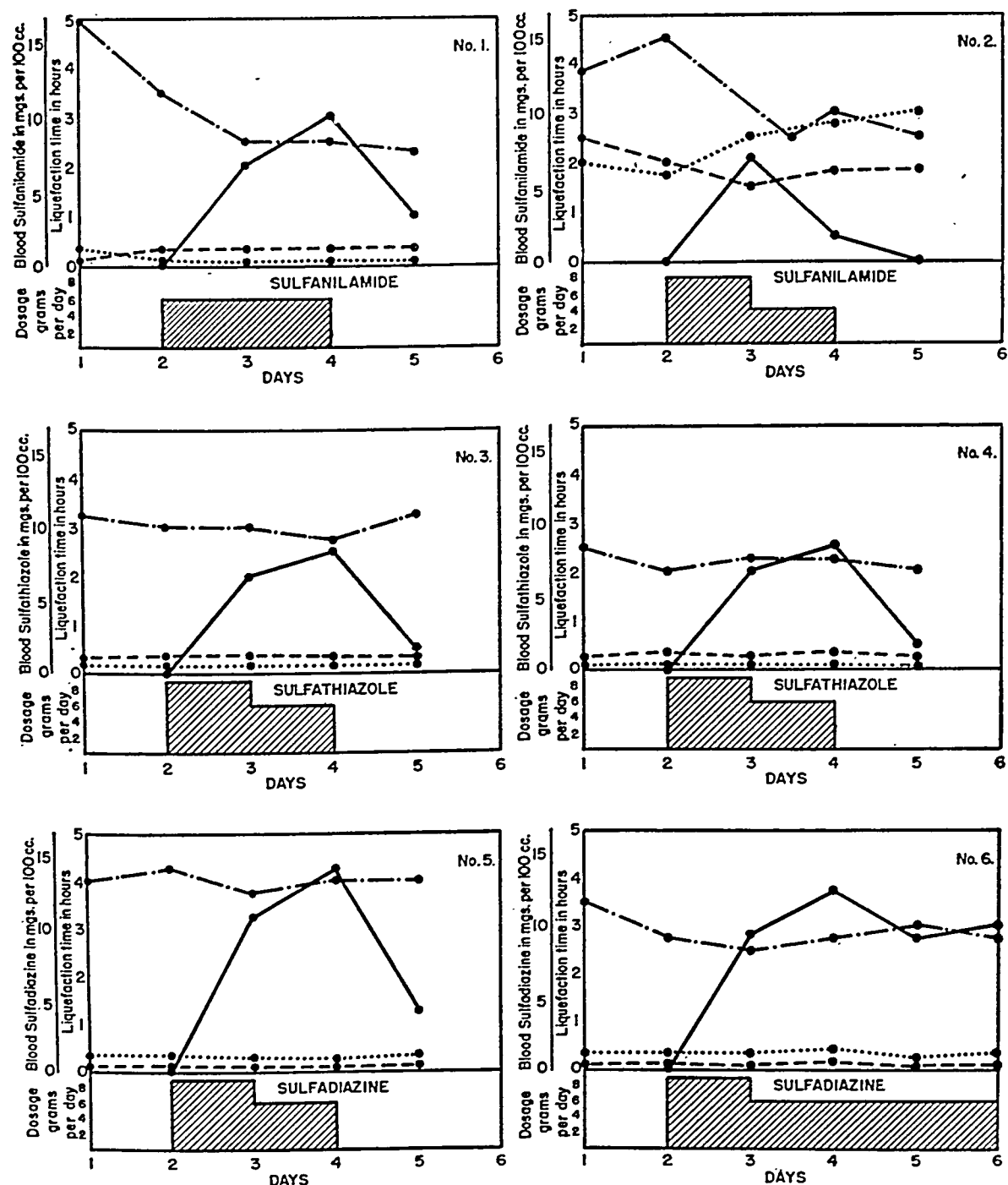
fibrinolysin times were the same as those shown on the chart.

COMMENT

Evidence is presented to show that sulfonamides have no effect whatsoever on the streptococcal antifibrinolysin test, either *in vitro* or *in vivo*. These results are not in accord with those of Hines, Hoover, and Graff (5) who found that, *in vivo*, sulfonamide administration caused an increased resistance to fibrinolysis.

The following explanation may account for these conflicting results: Massell, Mote, and Jones (9), in studying the quantitative aspects of the antifibrinolysin test, showed that, when the concentration of fibrinolysin is low, a very slight decrease in concentration will produce marked prolongation of the liquefaction time. For example, a liquefaction time of four hours can be easily increased to twenty-four hours by decreasing only slightly the concentration of fibrinolysin. Such a weak lytic strain was apparently used in the experiments of Hines *et al.*, for the initial liquefaction time was relatively long in every instance. Indeed, in spite of the statement that those with initial liquefaction times of over four hours were excluded, it can be seen from their protocols that two were in the neighborhood of five hours. Thus, any factor associated with sulfonamide administration which would only slightly decrease the fibrinolysin concentration would cause an apparent increased resistance to fibrinolysis. One such factor might be the inhibiting effect of the sulfanilamide in the patient's plasma upon the bacterial growth of the broth culture during the three or four hours required to perform the test. This might decrease the amount of fibrinolysin sufficiently to prolong markedly the liquefaction time. This would, of course, be an *in vitro* rather than an *in vivo* effect. The *in vitro* experiments of Huntington (7), and also those described here, were performed with more actively lytic strains, so that the results are not comparable. This explanation, if correct, is dependent upon a very weak lytic substance, since the amount of sulfonamide in the patient's plasma is small.

The Lancefield group C strain was actively lytic, comparable to the solid preparation of an active group A strain. Further studies of the immuno-



THIS FIGURE SHOWS THE RESULTS IN SIX PATIENTS, OF WHICH TWO RECEIVED SULFANILAMIDE, TWO SULFATHIAZOLE, AND TWO SULFADIAZINE. SOLID LINE REPRESENTS BLOOD LEVELS. LYTIC STRAINS ARE AS FOLLOWS; NO. 1. NO. 2. NO. 3.

FIG. 1. FAILURE OF SULFONAMIDE ADMINISTRATION TO EFFECT THE STREPTOCOCCAL ANTIFIBRINOLYSIN TEST *in Vivo*

logical properties of this organism will be presented elsewhere. The scarlet fever cases were of special interest. In them sulfonamide administration had no effect upon fibrinolysis but they later developed increased amounts of antifibrinolysin as a result of the hemolytic streptococcal infection.

SUMMARY AND CONCLUSIONS

Evidence is presented to show that sulfonamide administration, both *in vitro* and *in vivo*, has no effect upon the streptococcal antifibrinolysin test. The results of the *in vivo* experiments are not in agreement with those reported from another laboratory. The reasons for this discrepancy are discussed.

BIBLIOGRAPHY

1. Tillett, W. S., and Garner, R. L., The fibrinolytic activity of hemolytic streptococci. *J. Exper. Med.*, 1933, 58, 485.
2. Myers, W. K., Keefer, C. S., and Holmes, W. F., Jr., The resistance to fibrinolytic activity of the hemolytic streptococcus with special reference to patients with rheumatic fever and rheumatoid (atrophic) arthritis. *J. Clin. Invest.*, 1935, 14, 119.
3. Waaler, E., Development of antifibrinolytic properties in blood of patients with rheumatic fever, chronic infective arthritis, and bacterial endocarditis. *J. Clin. Invest.*, 1937, 16, 145.
4. Boisvert, P. L., The streptococcal antifibrinolysin test in clinical use. *J. Clin. Invest.*, 1940, 19, 65.
5. Hines, L. E., Hoover, A. H., and Graff, E., Effect of sulfanilamide on fibrinolytic activity of hemolytic streptococci. *Arch. Int. Med.*, 1940, 65, 744.
6. Garner, R. L., and Tillett, W. S., Biochemical studies on the fibrinolytic activity of hemolytic streptococci. 1. Isolation and characterization of fibrinolysin. *J. Exper. Med.*, 1934, 60, 239.
7. Huntington, R. W., Failure of sulphanilamide to prevent hemolysis, fibrinolysis, and production of erythrogenic toxin by hemolytic streptococci *in vitro*. *Proc. Soc. Exper. Biol. and Med.*, 1938, 38, 328.
8. Bratton, A. C., and Marshall, E. K., Jr., A new coupling component for sulfanilamide determination. *J. Biol. Chem.*, 1939, 128, 537.
9. Massell, B. F., Mote, J. R., and Jones, T. D., The quantitative relation of fibrinolysin and antifibrinolysin. *J. Immunol.*, 1939, 36, 45.

LATENT LIVER DISEASE IN PERSONS RECOVERED FROM CATARRHAL JAUNDICE AND IN OTHERWISE NORMAL MEDICAL STUDENTS AS REVEALED BY THE BILIRUBIN EXCRETION TEST

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The frequency with which chronic non-hemolytic jaundice occurs in otherwise healthy young persons is not commonly recognized, nor is it usually appreciated how often an uncomplicated attack of catarrhal jaundice is followed by residual liver dysfunction which persists long after the acute episode.

The present study was prompted by the accidental discovery of a relatively large number of medical students with chronic jaundice and a history of a previous attack of catarrhal jaundice in two of them. A group of persons long recovered from catarrhal jaundice was investigated and also a control group of persons presumably normal. The bilirubin excretion test was used in all cases because of its reliability in detecting lesser degrees of liver dysfunction.

METHODS

Bilirubin excretion test. The method of Von Bergmann and Eilbott (1, 2) was used with modifications. Bilirubin (Eastman Kodak) was given under basal conditions in a dose of 1.5 mgm. per kilogram of body weight. No untoward reactions were observed when fresh materials were used. A retention at 4 hours in excess of 15 per cent was regarded as abnormal.

Bilirubin determination, qualitative and quantitative. The photoelectrometric method of Malloy and Evelyn (3) was used. Hemolysis was avoided and analyses which failed to check within 2 per cent were rejected. The Evelyn colorimeter manual gives the range of normal serum values for direct bilirubin as 0.1 to 0.4 mgm. per cent and for indirect (total) bilirubin as 0.2 to 0.7 mgm. per cent. The ratio of the direct to indirect bilirubin is given clinical significance when elevated values occur. The ratio is high (0.60 to 0.80) in obstructive jaundice, intermediate (0.25 to 0.55) in diffuse parenchymal damage, and low (0.15) in hemolytic jaundice.

Bromsulphalein excretion test. A dose of 5 mgm. per kilogram of body weight was injected intravenously and a retention in excess of 10 per cent at 30 minutes was regarded as abnormal. **Galactose tolerance test.** Forty grams of the sugar were given orally; a urinary output of more than 3 grams within 5 hours after ingestion was

regarded as indicative of liver damage. **Cholecystogram.** A dose of tetraiodophenolphthalein was administered orally 18 and 12 hours before the test. **Fragility of red blood cells to hypotonic solutions of sodium chloride.** The method of Waugh and Asherman (4) was used with saline. Determinations were made with the Evelyn colorimeter. **Sedimentation rate.** The Wintrobe method was used. Values from 0 to 9 mm. per hour were regarded as normal for males. **Reticulocyte count.** The "wet" method was used. A drop of capillary ear blood was placed on a dry film of cresyl blue (in saturated alcohol solution), and the reticulocytes in 1000 red blood cells were counted. The normal range was considered to be 0 to 2.5 per cent. **Urobilin.** The Schlessinger reaction was used for the qualitative determination of urobilin in the urine. A definite fluorescence in the filtrate was a positive test. **Urobilinogen.** Ehrlich's aldehyde method was employed for the detection of excess urobilinogen in the urine. A red color in a dilution greater than 1:10 was a positive test. **Vitamin A absorption test and plant pigment determination.** The method of Chesney and McCoord was used. Fish liver oil containing 7000 U.S.P. vitamin A units for each kilogram of body weight was given. The vitamin A content of the blood was measured by the McCoord and Luce-Clausen (5) modification of the Carr and Price technique. The analysis for the carotenoids or plant pigments (xanthophyll and carotene), which was necessary for the correction of vitamin A results, was done by the method of Clausen and McCoord (6).

RESULTS

1. *Persons presumed to be normal.* For this group, subjects were selected who gave no indication of liver dysfunction by history or physical examination. They were in the younger age groups and were recovering from minor surgical operations.

The basal serum bilirubin concentrations which were generally below 0.8 mgm. per cent are in Table I, and the individual values during the excretion test are in Table II. Of 22 persons tested, 95 per cent retained less than 15 per cent of the injected bilirubin and 73 per cent retained less than 10 per cent. The sole person who retained

TABLE I

Distribution of basal bilirubin values in persons presumed to be normal

Range: 0.114 mgm. per cent to 0.900 mgm. per cent
 Mean concentration: 0.398 mgm. per cent
 Standard deviation: 0.184 mgm. per cent

Serum bilirubin mgm. per cent	Number of cases	Per cent of total
0.000-0.199	3	10
0.200-0.399	11	38
0.400-0.599	12	41
0.600-0.799	2	7
0.800-0.999	1	4
Total	29	100

TABLE II

Individual bilirubin excretion test values in persons presumed to be normal

Per cent retention at 240 minutes

$$= \frac{C_{240 \text{ minutes}} - C_0 \text{ minutes}}{C_5 \text{ minutes} - C_0 \text{ minutes}} \times 100$$

Case number	Age	Serum bilirubin						Per cent retention at 240 minutes
		0 min-utes	5 min-utes	30 min-utes	60 min-utes	90 min-utes	240 min-utes	
	years	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	
1	5	0.298	2.430	1.330	0.881	0.663	0.503	9.4
2	29	0.114	2.140	1.200	0.628	0.522	0.282	8.4
3	28	0.114	2.610	1.220	0.700	0.556	0.332	8.8
4	31	0.264	2.810	1.240	0.863	0.628	0.503	9.4
5	16	0.400	3.250	1.560	1.010	0.790	0.538	4.9
6	16	0.645	3.920	2.400	1.590	1.300	0.975	10.1
7	14	0.522	3.860	2.160	1.360	1.070	0.682	4.8
8	13	0.592	4.200	2.450	1.630	1.320	0.900	8.6
9	13	0.418	3.060	1.520	0.938	0.718	0.645	8.7
10	20	0.574	3.770	2.100	1.440	1.090	0.918	10.6
11	14	0.490	3.350	2.030	1.340	1.070	0.790	10.5
12	23	0.430	3.330	2.030	1.430	1.110	0.830	13.8
13	19	0.131	2.380	1.010	0.592	0.434	0.204	3.6
14	23	0.500	3.120	1.590	1.010	0.790	0.540	1.5
15	20	0.214	3.460	1.610	1.002	0.790	0.522	9.6
16	33	0.282	3.060	1.740	1.010	0.700	0.452	6.1
17	25	0.503	4.610	2.590	1.730	1.350	0.955	11.0
18	23	0.900	4.320	2.960	2.430	2.080	1.560	19.6
19	24	0.538	3.770	2.170	1.420	1.120	0.790	7.7
20	22	0.347	2.060	1.080	0.760		0.450	5.9
21	20	0.330	2.780	1.420	0.880		0.540	8.6
22	20	0.420	3.240	1.460	0.810		0.560	5.0
23	7	0.247		1.090	0.647	0.469		
24	5	0.214		1.078	0.594	0.358		
25	5	0.307		1.550	1.097	0.857		
26	9	0.264		1.710	1.024	0.724		
27	10	0.264		1.540	1.070	0.770		
28	14	0.486		1.840	1.280	0.975		
29	21	0.718		2.120	1.520	1.200		

in excess of 15 per cent of the injected bilirubin was the one who had the extreme basal bilirubin value of 0.900 mgm. per cent. Mean bilirubin values at various time intervals during the test are

TABLE III

Mean bilirubin excretion test values in persons presumed to be normal and others long recovered from catarrhal jaundice

The values at 0 minutes are the basal bilirubin levels. $C_i - C_0$ = Concentration at time, "i," minus basal bilirubin concentration, "0". The mean bilirubin values are followed by their standard errors. The mean values for the two groups were significantly different at each time period except the one at 5 minutes.

Time	"Normal" group			"Post-catarrhal jaundice" group		
	Number of cases	Mean value ($C_i - C_0$)	Coef- ficient of vari- ation	Number of cases	Mean values ($C_i - C_0$)	Coef- ficient of vari- ation
min- utes						
0	29	0.396±0.034	0.460	17	0.616±0.061	0.408
5	22	2.760±0.106	0.181	16	2.940±0.080	0.109
30	29	1.320±0.065	0.268	17	1.650±0.085	0.212
60	29	0.710±0.048	0.366	17	1.120±0.085	0.312
90	26	0.490±0.039	0.408	17	0.870±0.085	0.402
240	22	0.240±0.030	0.584	17	0.540±0.073	0.506

in Table III. Figure 1 contains a composite curve of these mean bilirubin values, and Figure 2 shows the linear relationship which is obtained when these values are plotted against the logarithm of time.

2. *Persons long recovered from catarrhal jaundice.* The subjects for this group were chosen completely at random from the hospital records of adequately studied and confirmed cases of acute catarrhal jaundice. They were generally children or young adults who, during their acute episode of jaundice, had symptoms of malaise, nausea, and abdominal pain. There was a sudden onset of jaundice which often coincided with symptomatic improvement. A familial incidence was noted in half of the cases. Fever, hepatomegaly, leukopenia with an increase in the percentage of mononuclear cells, bilirubinuria, and acholic stools were found in most cases. Hyperbilirubinemia was always present with an immediate direct van den Bergh reaction when tested. Treatment was symptomatic and recovery in each case was rapid and uneventful, with a prompt fall of the serum bilirubin level to near normal limits.

Individual serum bilirubin values during the excretion test are shown in Table IV, and the mean values in Table III. The latter have been shown to differ significantly from those for the "normal" group. Of 16 persons tested, 63 per cent retained more than 15 per cent of the in-

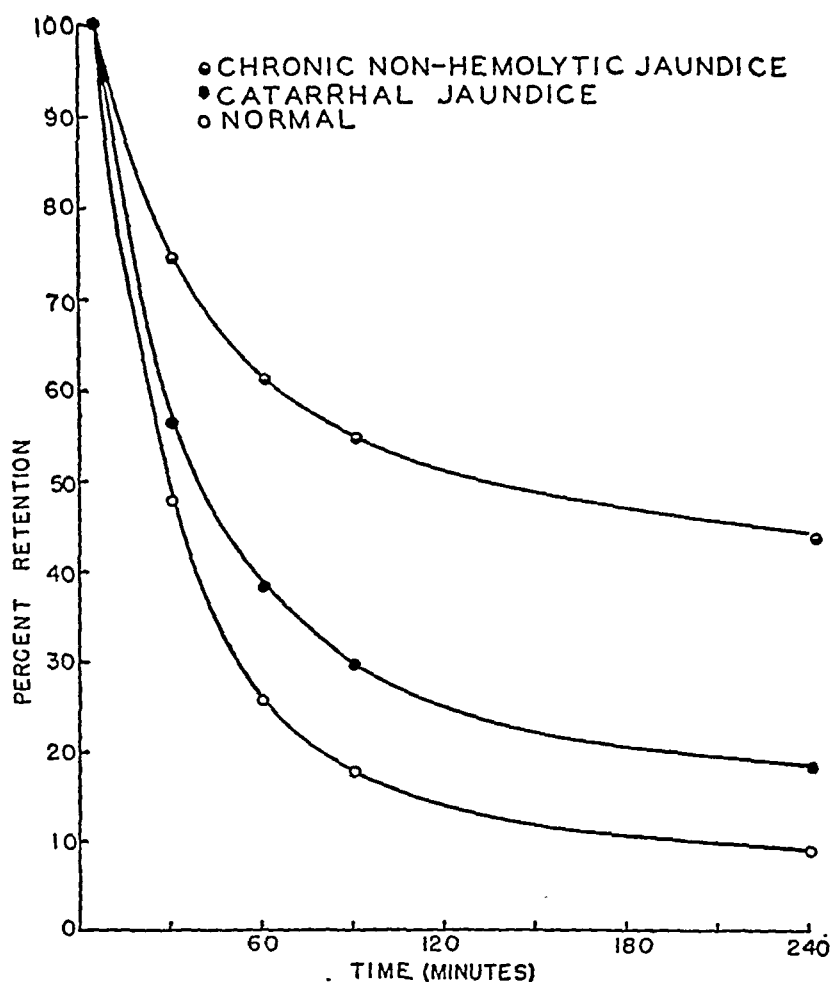


FIG. 1. COMPOSITE CURVES OF MEAN SERUM BILIRUBIN VALUES DURING THE EXCRETION TEST

jected bilirubin and 95 per cent retained more than 10 per cent. Composite curves for the data in Table III are in Figures 1 and 2.

Among the 10 persons who showed an abnormal bilirubin excretion curve, a history at the time of the test revealed 4 with symptoms suggestive of liver dysfunction (Cases 30, 33, 40, 45), 3 with only vague abdominal pains or anorexia (Cases 35, 37, 44), and 3 without any symptoms (Cases 31, 32, 36). The pertinent clinical data of 5 of these 10 patients are presented here.

Case 30. A 12-year-old boy has been followed in the pediatric clinic since the age of 6 years for enuresis, habit spasms, and temper tantrums. His father has a convulsive disorder, and the home environment is unstable. In October 1940 he had an attack of catarrhal jaundice and was discharged after 5 days. In the Out-Patient Depart-

ment he complained of anorexia, fatigue, malaise, right upper quadrant tenderness, and mild epigastric pain. Four months after discharge he was readmitted to the hospital for severe paraumbilical pain, nausea, vomiting, marked distension, and foul flatus. The only positive findings were a flat glucose tolerance curve and an abnormal excretion of bilirubin despite normal basal bilirubin values. Supportive care eased the symptoms, and he was discharged with the diagnosis of aerophagia. He continued to be irritable, became fatigued easily, and complained of diffuse abdominal pain, nausea, flatulence, headaches, and severe constipation. Two weeks later he returned to the hospital with frank jaundice, an enlarged tender liver, and a readily palpable spleen. Again complete hematological, chemical, and x-ray studies were entirely negative, except for a basal bilirubin level of 1.22 mgm. per cent and a bilirubin retention of 41.8 per cent. Other tests of liver function were repeatedly negative. After a 2-week dietary regime of low fat and high car-

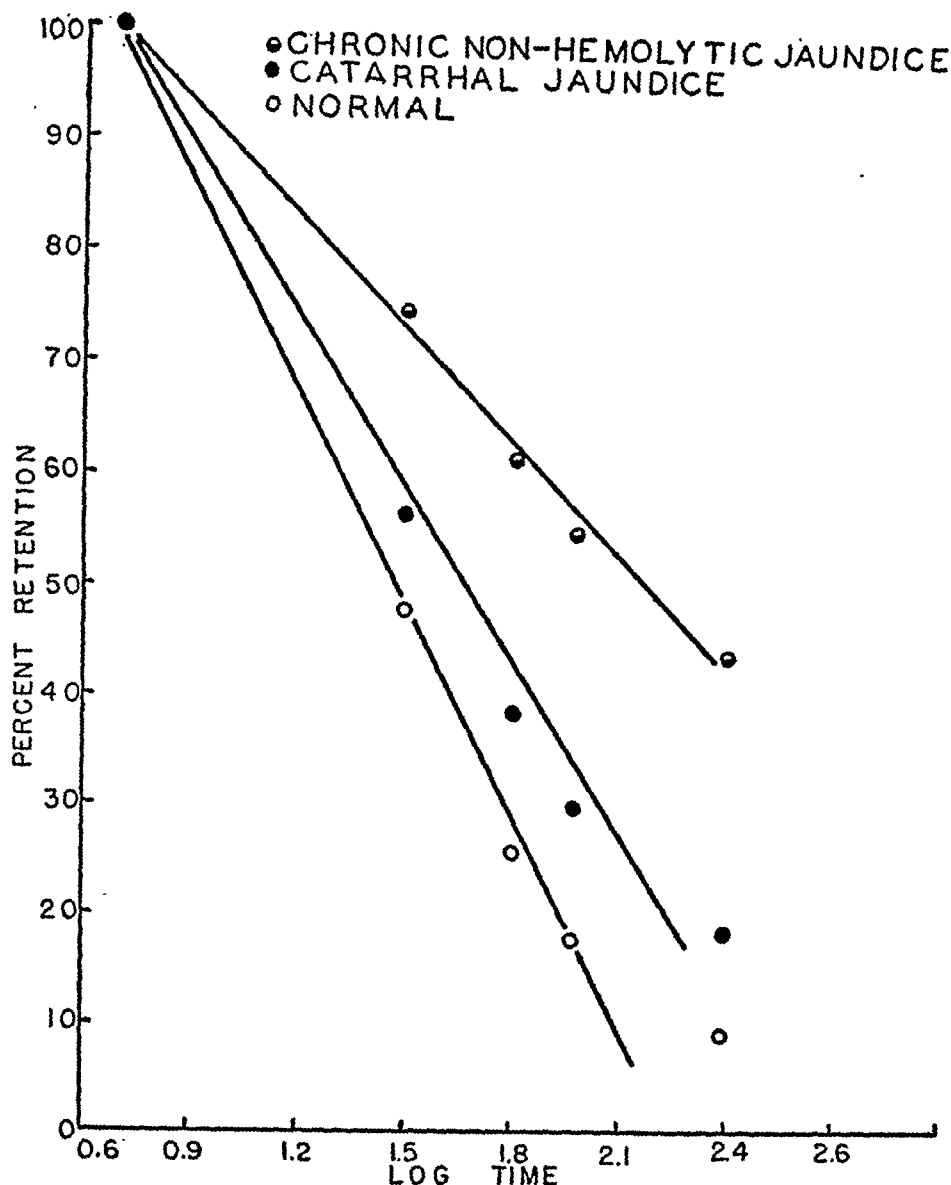


FIG. 2. COMPOSITE CURVES OF MEAN SERUM BILIRUBIN VALUES DURING THE EXCRETION TEST PLOTTED AGAINST THE LOGARITHM OF TIME

bohydrate and protein intake, he was discharged as improved.

Case 31. This 49-year-old laborer had catarrhal jaundice in August 1940. A gastro-intestinal series revealed a tender, incompletely filled duodenal cap; other studies were negative. Eight months later his basal bilirubin level was 1.22 mgm. per cent with a D/I (direct:indirect) ratio of 0.38 and a 4-hour bilirubin retention of 47.8 per cent. His liver was firm and palpable 5 cm. below the costal margin, but he was asymptomatic. He admitted moderate alcohol ingestion.

Case 33. This 20-year-old male has been studied in this clinic since the age of 14. His most frequent complaints were malaise and shifting abdominal pains. In September 1938 he was hospitalized for a 2-day episode of unexplained diarrhea with high fever and in April 1940 for catarrhal jaundice. Three months later he returned to the Out-Patient Department with complaints of epigastric distress and flatulence. A gastro-intestinal series revealed a slightly irritable cap. The diagnosis was

psychoneurosis. One year after his episode of jaundice, he still complained of malaise, anorexia, flatulence, irritability, and persistent pain in the upper abdominal quadrants. The basal bilirubin level was 0.860 mgm. per cent with a D/I ratio of 0.31 and a 4-hour retention of 21.1 per cent. A gallbladder series was normal.

Case 40. This 29-year-old hospital orderly had catarrhal jaundice in September 1939. He had residual belching, flatulence, abdominal distension, and a dull aching pain in the right upper quadrant. He could obtain no medical relief and resorted to herb remedies. He felt irritable and tired, and drank moderate amounts of alcohol. One and a half years after his jaundice, the basal bilirubin level was 0.520 mgm. per cent, and he still retained 18.2 per cent of the injected bilirubin at 4 hours. His liver was tender and palpable 2 cm. below the costal margin.

Case 45. This 16-year-old boy had catarrhal jaundice in February 1938. He visited the Out-Patient Department a few months after his recovery with complaints

TABLE IV

Individual bilirubin excretion test values in persons long recovered from catarrhal jaundice, March 1941

The basal bilirubin values ranged from 0.264 to 1.280 mgm. per cent with a mean value of 0.616 mgm. per cent and a standard deviation of 0.252 mgm. per cent.

Case number	Age	Interval since catarrhal jaundice	Serum bilirubin						Per cent retention at 240 min.
			0 minutes	5 minutes	30 minutes	60 minutes	90 minutes	240 minutes	
	years	months	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	
30(2/2)	11	4	0.628	3.040	2.490	2.030	1.950	1.560	37.6
(2/7)			0.503	3.360	2.360	1.860	1.580	1.200	24.5
(3/1)			1.280	5.090	3.920	3.520	3.220	2.870	41.8
31	49	8	1.220	4.170	3.180	2.870	2.490	2.630	47.8
32	10	10	0.682	3.950	2.890	2.300	1.970	1.360	20.8
33	19	11	0.863	3.980	2.780	2.100	1.990	1.520	21.1
34	12	13	0.332	2.990	1.590	0.992	0.808	0.610	10.5
35	23	13	0.660	3.550	2.540	1.930	1.660	1.140	16.6
36	13	13	0.660	3.120	2.030	1.690	1.380	1.180	21.1
37	15	14	1.010	3.740	2.910	2.570	2.400	1.650	23.4
38	20	16	0.682	3.430	2.120	1.400	1.180	1.030	12.7
39	13	16	0.522	3.400	2.030	1.420	1.190	0.881	12.8
40	29	18	0.520	3.710	2.240	1.820	1.380	1.100	18.2
41	7	23	0.400		1.360	0.920	0.770	0.522	
42	8	25	0.231	2.490	1.320	0.920	0.700	0.452	9.7
43	15	24	0.574	3.580	2.260	1.510	1.190	0.937	12.3
44	11	26	0.264	2.870	1.710	1.240	1.010	0.736	18.2
45	15	38	0.503	3.660	2.380	1.670	1.460	1.010	16.1
46	22	42	0.556	3.630	2.260	1.630	1.440	0.937	12.4

of fatigue, abdominal and chest pain, headaches, severe constipation, and inability to attain his former proficiency at school. Three years later the basal bilirubin level was 0.503 mgm. per cent, and he retained 16.1 per cent of the injected pigment at 4 hours. The above mentioned symptoms were persistent.

3. Persons with chronic non-hemolytic jaundice.

This group consists of 7 medical students and 1 graduate chemistry student (Case 54). It arose as a result of casual observations of less than 100 medical students and a selection of those who appeared to have an icteric tint to their sclerae. The laboratory studies of these individuals revealed the basal bilirubin level to be consistently elevated over a period of many months with a van den Bergh reaction indirect in type. The bilirubin excretion was markedly retarded in every instance, and in several individuals other tests of liver function yielded positive results. The hematological, x-ray, blood chemical, and urinary studies were uniformly negative. In Table V are the assembled data for this group, and in Figures 1 and 2 the composite bilirubin excretion curves are shown. Figure 3 contains the results of vitamin A absorption tests.¹

¹At present a further group is being investigated. Among the first and second year medical classes, comprising approximately 120 persons, routine icterus indices

Some interesting data were obtained from clinical histories and physical examinations of these 8 individuals and they are summarized here.

Case 47. This 26-year-old male had catarrhal jaundice in September 1938. His icterus index upon discharge was 18 units. In October 1939 he had unexplained abdominal cramps and diarrhea for several days and a palpable liver and spleen. In October 1940 his basal bilirubin level was 1.5 mgm. per cent, he was asymptomatic, and his liver and spleen were no longer palpable.

Case 48. This 25-year-old male, at the age of 8, had the "yellow jaundice." At the age of 17 he was exposed to carbon tetrachloride for 6 weeks, while working in a cleaning plant. He had drunk raw milk, and his agglutinin titre for *Brucella melitensis* was 1:80 in 1938. In 1939, he had a tuberculous pleural effusion with recovery, following a year of sanatorium care. Subicteric sclerae were noted in January 1940. In October 1940 his basal bilirubin level was 1.36 mgm. per cent.

Case 49. This 29-year-old male recalled having had "dirty-looking" eyes for many years. At the age of 19 he worked in a gasoline filling station for one year. At 21 years he had acute appendicitis, generalized peritonitis, pelvic abscess, and finally recovered without re-

vealed 10 with elevated values. Bilirubin excretion tests on 6 of these persons, performed by Dr. E. B. Millard, Jr., demonstrated basal bilirubin values of 1.45, 1.59, 1.62, 1.63, 1.67, and 1.75 mgm. per cent with 4-hour retention percentages of 79.2, 70.7, 58.9, 70.9, 31.5, and 47.9 respectively.

TABLE V
Results of the bilirubin excretion test and other studies in persons with chronic non-hemolytic jaundice, March 1941

Case number	Bilirubin excretion test										Graham test of gallbladder function	Blood chemistry							Hematology							Urine																																																																																																																																																																																																																																																																																																																																																																													
	Serum bilirubin							Per cent retention		Bromsulphalein test per cent retained at 30 minutes		Galactose tolerance test grams excreted in 5 hours	Plasma proteins 1	Serum albumin	Ratio of serum albumin to globulin	Non-protein nitrogen	Fibrinogen	Cholesterol	Xanthophyll	Carotene	Red blood cells	Hemoglobin (Sabin)	White blood cells	Differential cell count and smear	Reticulocyte count			Hematocrit	Sedimentation rate	Fragility test of red blood cells																																																																																																																																																																																																																																																																																																																																																																									
	0 min-utes	5 min-utes	30 min-utes	60 min-utes	90 min-utes	240 min-utes	24 hours	mgm. per cent	mgm. per cent																						mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per

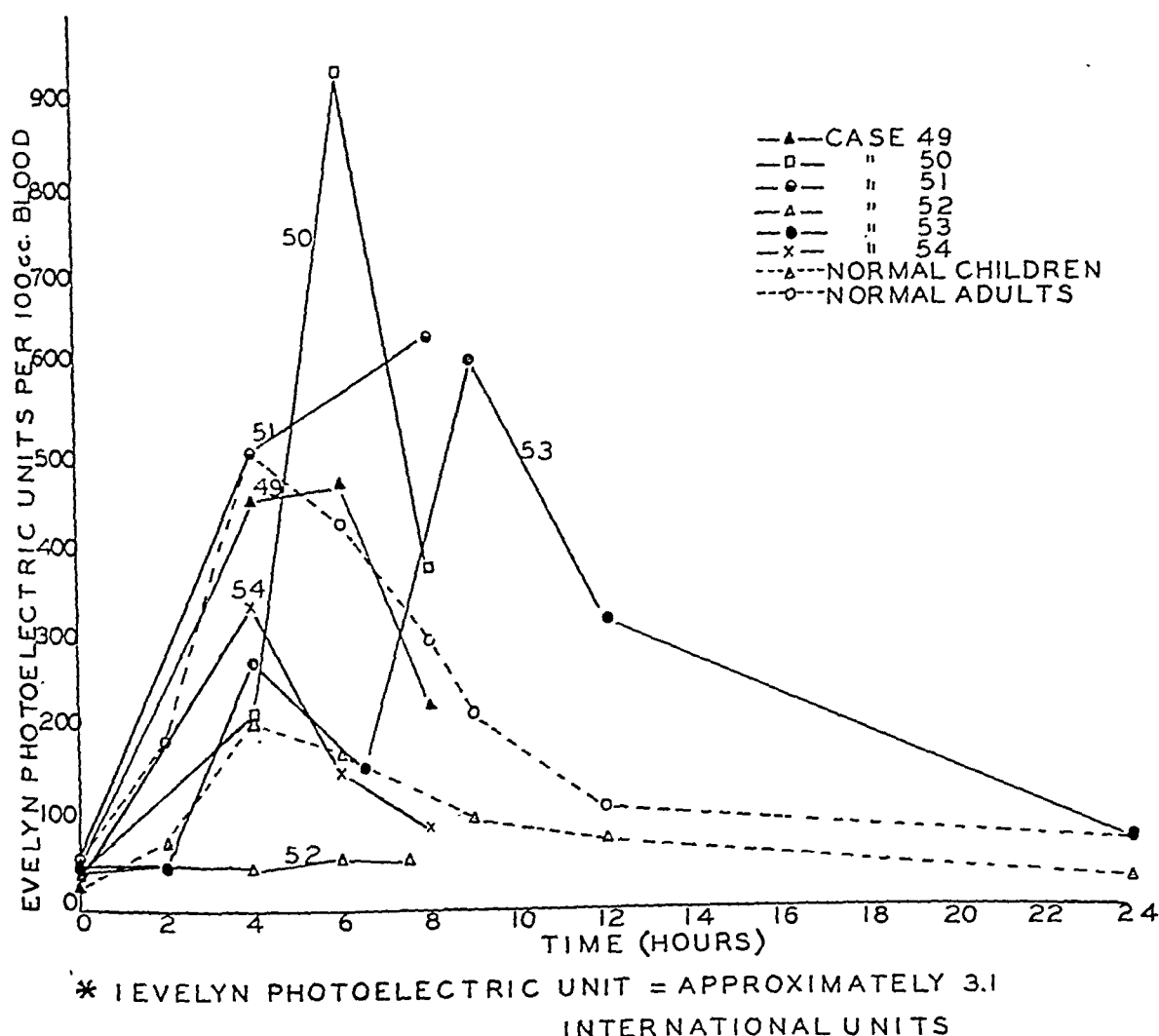


FIG. 3. VITAMIN A ABSORPTION TEST

sidua. Subicteric sclerae were noted in 1939. In October 1940 his icterus index was 14 units.

Case 50. This 26-year-old male had jaundice neonatally and once or twice in childhood. Since 1938 he has had flatulence, constipation, occasional vomiting, and generalized discomfort after eating. These symptoms are aggravated by emotional upsets. He has chronic sinusitis. In October 1940 his icterus index was 14 units.

Case 51. This 25-year-old male had typhoid fever at 5 years. The icteric tint of his sclerae was noted in October 1940. In March 1941 his liver was tender and palpable 3 cm. below the costal margin. He complained of occasional drawing right upper quadrant pains. A maternal grandmother died of obstructive jaundice.

Case 52. This 25-year-old male at 16 years aspirated a mercurial solution. This was followed by severe diarrhea for several days and then recovery. At 19 he had an appendectomy for chronic abdominal pain with some

relief. At 20 a physician casually observed his sclerae to be yellow. The jaundice has been more notable during exacerbations of sinus disease. In April 1940 his icterus index was 22 units. All diagnostic procedures directed at the etiology of his jaundice during a hospital study were negative. Blood bilirubin levels of his family showed normal values for all but his mother, who had a level of 1.26 mgm. per cent with a D/I ratio of 0.36. A maternal grandmother died of hepatic cirrhosis at 72 years after a lifetime of jaundice.²

Case 53. A 23-year-old male, whose jaundice was noted in November 1939, had at that time an icterus index of 14 units. He complained of flatulence, a partial

² A bilirubin excretion test in November 1941 revealed a basal value of 2.25 mgm. per cent with a 4-hour retention of 64.3 per cent as compared with 61.2 per cent in March 1941.

TABLE VI

Individual bilirubin excretion test values in persons with miscellaneous conditions

Case number	Age	Diagnosis	Serum bilirubin						Per cent retention at 240 minutes
			0 minutes	5 minutes	30 minutes	60 minutes	90 minutes	240 minutes	
	years		mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	mgm. per cent	
61	60	Carcinoma of pancreas	4.550	6.920	5.920	5.310	5.190	4.980	18.2
62	18	Hodgkin's disease	0.538	2.170	1.390	1.080	0.920		
64	15	Lymphosarcoma	0.366	2.490	1.230	0.753	0.592	0.556	8.2
65	59	Lymphosarcoma	0.500	3.300	1.990	1.300	1.090	0.880	13.5
66	44	Chronic lymphatic leukemia	0.503	2.700	1.820	1.360	0.992	0.863	16.3
67	65	Chronic myelogenous leukemia	0.468	2.610	1.500	0.968	0.835		
68	55	Carcinoma of liver	0.592	2.990	1.860	1.320	1.140		
69	57	Portal cirrhosis	4.840	7.180	6.790	6.200	5.880	5.340	21.4
70	67	Portal cirrhosis	0.736	3.920	2.440	1.850	1.610	1.260	16.4
71	10	Paroxysmal hemoglobinuria	0.418	2.780	1.910	1.400	1.090	0.881	19.5
72	36	Hepatitis, convalescent	0.592	3.090	1.650	1.090	0.937	0.825	9.6

intolerance to fatty and fried foods, occasional shifting abdominal pains, and several episodes of unexplained diarrhea. Icterus levels were followed closely during the next year and varied between 10 and 20 units. Repeated x-rays of the gallbladder and intestinal tract, and hematological and blood chemical studies were negative. His mother died of an operative complication of cholecystectomy for chronic cholecystitis and cholelithiasis. A sister has symptoms of gallbladder disease. A maternal aunt had a cholecystectomy many years ago and is jaundiced at present.

Case 54. Jaundice was discovered in this 24-year-old male during the routine blood chemical studies of individuals doing research with arsenical gases. Traces (1 gamma or less) of arsenic were found in the urine, hair, blood serum, and cells. Cadmium appeared as a trace in the urine, in a concentration of 3 gamma in the hair, and 10 gamma per cc. in the blood serum. He remembered volatilizing cadmium and sulphur in a crucible 1 year ago. There was no known exposure to free arsenical fumes.

4. Persons with miscellaneous conditions. The bilirubin excretion test was performed on a varied group of persons suspected of liver dysfunction. Positive results were obtained in 1 person with chronic leukemia, 2 persons with portal cirrhosis, and in 1 person with paroxysmal hemoglobinuria. Bilirubin excretion within normal limits occurred in 2 persons with lymphosarcoma and in 1 person recovering from jaundice coincident with pneumonia.

DISCUSSION

Observations of 29 presumably normal individuals indicate that the basal serum bilirubin level is generally below 0.8 mgm. per cent. Bilirubin injected in a dose of 1.5 mgm. per kilogram

of body weight is excreted by these subjects in a relatively uniform way, the retention at 4 hours after injection never exceeding 15 per cent. A comparison of these results with those in the literature is difficult because of the diverse techniques employed.

A group of individuals, otherwise random, who were long recovered from a well-studied attack of catarrhal jaundice, differed significantly from these normal subjects. Of 16 individuals tested, 10 gave evidence of abnormal excretion of bilirubin and 4 of these 10 described symptoms of liver dysfunction, which prior to the test had been unexplained. Their complaints were vague abdominal pains, flatulence, anorexia, and general malaise. Hepatomegaly and jaundice occurred in several individuals. These laboratory and clinical findings are in close agreement with those described by Kalk (7) and Soffer (8) and serve to emphasize, along with the more recent concept of the pathogenesis of catarrhal jaundice, that this disease is not so completely benign as it is commonly regarded. Rather it is seen that long-lasting impairment of liver function, often associated with a suggestive symptom complex, is a frequent residuum of an uncomplicated episode of catarrhal jaundice.

Eight cases of chronic non-hemolytic jaundice were discovered in a small body of medical students. Comparable experiences have been described by others. Polack (9) found 8 cases of jaundice in young persons which he ascribed to chronic hepatitis resulting from antecedent acute

episodes. Meulengracht (10) saw 24 persons between the ages of 15 and 30 years who had mild chronic jaundice. He regarded the abnormality as a benign hepatic disorder rather than hemolytic jaundice, chronic hepatitis, or "physiological hyperbilirubinemia." A high incidence of medical students and physicians was noted in these groups, and it was attributed to the greater acuity of these persons in detecting slight degrees of jaundice.

A careful study of the individuals in the present series revealed a uniformly marked inability to excrete intravenously injected bilirubin. Galactose was excreted in excessive amounts in several members of the group, and one subject was unable to absorb ingested vitamin A. Bromsulphalein, x-ray, hematological, blood chemical, and urinary studies were negative. The etiology and the precise nature of these disorders are not clear. Probably they are examples of low-grade parenchymal dysfunction which have arisen from one or several causes, such as exposure to poisons or inflammatory disease.

Soffer (11) and others have stated that the bilirubin excretion test is contraindicated in the presence of hyperbilirubinemia, because of the manifest inability of the individual to excrete bilirubin. While these theoretical objections may possibly hold in purely obstructive types of jaundice, they do not apply with equal force to other types of jaundice. In the present study useful information has been derived from the application of the test to persons with low-grade chronic non-hemolytic jaundice. Methods of calculation (Weech *et al.*, 12) designed to eliminate the effect of elevated basal bilirubin levels, yield results, in this group of individuals, which do not differ from those reported here with the use of the conventional retention formula, originally proposed by Von Bergmann and Eilbott (1, 2). Also there are several reports (13, 2, 7, 14) of normal excretion in the presence of jaundice.

Von Bergmann and Eilbott found the rate of bilirubin excretion to be proportional to the concentration in the blood at any particular time. This relationship was not obtained from the present data. From the linear relation of the serum bilirubin values to the logarithm of time (Figure 2), it can be inferred that within limits the excretory rate varies inversely with time. Although

simple, this relation gives little information about the kinetics of bilirubin removal.

Weech *et al.* (12) propose a formula for the measurement of bilirubin excretion based on the assumption that within certain defined limits the excretory rate is approximately proportional to the square of the plasma concentration. This formula was applied to the data of the present investigation, and the results were compared with those obtained with the conventional retention formula. The coefficient of correlation, "r" (product moment), for the entire body of data was 0.68 and that for the group with chronic non-hemolytic jaundice, separately, was 0.86.

Dragstedt and Mills (15) in studying the removal of intravenously injected bilirubin in dogs were puzzled by the low concentrations present in the serum 5 minutes after injection. They obtained only 40 per cent of the concentration calculated to be there if the material were distributed solely in the plasma, whereas in the present studies of human subjects these 5-minute values were usually close to 100 per cent. These workers (16) also concluded as a result of reticulo-endothelial cell blockade experiments in dogs, that bromsulphalein retention occurred whenever jaundice was present. However, the group of individuals with chronic non-hemolytic jaundice in this study showed no pathological retention of bromsulphalein.

CONCLUSIONS

1. Persons long recovered from catarrhal jaundice show an abnormal retention of bilirubin and possess symptoms of liver dysfunction in a considerable percentage of the cases.

2. Chronic non-hemolytic jaundice occurs in otherwise healthy young persons much more frequently than has been commonly recognized.

3. Presumably normal persons have basal bilirubin values which fall within a narrow range and excrete intravenously injected bilirubin in a relatively uniform way.

4. The bilirubin excretion test is a reliable and sensitive test of liver function.

I wish to express my gratitude to Professor William S. McCann whose encouragement made this work possible. I am also indebted to Mr. A. V. Wolf for his helpful advice throughout the course of this work.

BIBLIOGRAPHY

1. Von Bergmann, G., Zur Funktionellen Pathologie der Leber insbesondere der Alkohol-Ätiologie der Cirrhose. *Klin. Wchnschr.*, 1927, 6, 776.
2. Eilbott, W., Funktionsprüfung der Leber Mittels Bilirubinbelastung. *Ztschr. f. klin. Med.*, 1927, 106, 529.
3. Malloy, H. T., and Evelyn, K. A., The determination of bilirubin with the photoelectric colorimeter. *J. Biol. Chem.*, 1937, 119, 481.
4. Waugh, T. R., and Asherman, E. G., The use of an index of hemolysis in expressing the fragility of erythrocytes. *J. Lab. and Clin. Med.*, 1938, 23, 746.
5. McCoord, A. B., and Luce-Clausen, E. M., The storage of vitamin A in the liver of the rat. *J. Nutrition*, 1934, 7, 557.
6. Clausen, S. W., and McCoord, A. B., The determination of carotene and xanthophyll by a single distribution between liquid phases. *J. Biol. Chem.*, 1936, 113, 89.
7. Kalk, H., Klinische Untersuchungen über die Frage des latenten Leberschadens. I. Untersuchungen mit der Bilirubinbelastungsprobe beim Ikterus Catarrhalis. *Deutsche med. Wchnschr.*, 1932, 58, 1078; II. Klinische Untersuchungen bei den sogenannten Hepatopathien nach überstandenen Ikterus Catarrhalis. *Ibid.*, 1932, 58, 1119.
8. Soffer, L. J., and Paulson, M., Residual hepatic damage in catarrhal jaundice as determined by the bilirubin excretion test. *Arch. Int. Med.*, 1934, 53, 809.
9. Polack, E., Chronic hepatitis in young persons with or without intermittent jaundice. *Acta med. Scandinav.*, 1938, 93, 614.
10. Meulengracht, E., Icterus Intermittens Juvenilis (Chronischer Intermittierender Juveniler Subikterus). *Klin. Wchnschr.*, 1939, 18, 118.
11. Soffer, L. J., and Paulson, M., Comparative advantages and further modifications of the bilirubin excretion test for hepatic function. *Am. J. M. Sc.*, 1936, 192, 535.
12. Weech, A. A., Vann, D., and Grillo, R. A., The clearance of bilirubin from the plasma. A measure of the excreting power of the liver. *J. Clin. Invest.*, 1941, 20, 323.
13. Damashek, W., and Singer, K., Familial nonhemolytic jaundice. *Arch. Int. Med.*, 1941, 67, 259.
14. Lin, H., and Eastman, N. J., The behavior of intravenously injected bilirubin in newborn infants. *Am. J. Obst. and Gynec.*, 1937, 33, 317.
15. Dragstedt, C. A., and Mills, M. A., The removal of intravenously injected bilirubin from the blood stream in the dog. *Am. J. Physiol.*, 1937, 119, 713.
16. Dragstedt, C. A., and Mills, M. A., Bilirubinemia and bromsulphalein retention. *Proc. Soc. Exper. Biol. and Med.*, 1936, 34, 467.

VITAMIN A DEFICIENCY IN LAENNEC'S CIRRHOSIS. THE RELATIVE SIGNIFICANCE OF THE PLASMA VITAMIN A AND CAROTENOID LEVELS AND THE DARK ADAPTATION TIME¹

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Recent studies (1 to 9) have reported the occurrence of vitamin A deficiency in patients having cirrhosis of the liver. It has also been shown that a lowering of the amount of vitamin A available to the tissues may result from reduced vitamin A intake (10 to 14), and from faulty gastro-intestinal absorption (6, 15, 16), especially when associated with jaundice (16). It is known, moreover, that febrile states reduce the level of vitamin A in the blood (17, 18). Since both faulty gastro-intestinal absorption and fever (19) may occur in Laennec's cirrhosis, it is essential in a study of vitamin A deficiency in relation to this disease that these factors be controlled. In many of the published studies of the subject, either the appropriate controls are lacking, or the conditions bearing on these factors are unspecified.

In previous papers data were presented which revealed that (a) patients with cirrhosis of the liver may have greatly delayed dark adaptation which is improved by massive daily doses of vitamin A (3, 4); (b) there is no evidence in such patients of a causal relation between the level of vitamin A in the blood and the dark adaptation time (20); (c) the vitamin A concentration in the liver of rats with carbon tetrachloride cirrhosis was half that of normal rats fed the same amount of food and of vitamin A (21).

It is our present purpose to compare the dark adaptation time and the blood plasma concentration of vitamin A and of total carotenoid found in patients with Laennec's cirrhosis with those found in normal persons and in patients with certain other diseases.

METHODS

Dark adaptation measurements and determinations of the concentration of vitamin A and of total carotenoid in

¹ Preliminary accounts of these measurements were presented to the American Society of Zoologists in December, 1941 (Anat. Rec., 1941, 81, Suppl., 123, 124).

the blood plasma and in the liver were made by methods described elsewhere (20). Plasma vitamin A levels were expressed as international units (I. U.) per 100 ml. of plasma, plasma carotenoid levels as micrograms (μ gm.) per 100 ml. of plasma, and liver vitamin A and carotenoid contents as I.U. and μ gm., respectively, per gram of fresh tissue. The parameter of the dark adaptation function employed for comparison among the various subjects, called the *dark adaptation time*, is defined as the time in minutes required for the visual threshold to attain a value of 5.50 (in log micromicrolamberts). This quantity records variations in either the rate of adaptation or in the threshold level (20). The concentration of total cholesterol in the plasma was also determined, using the method of Bloor, Pelkin, and Allen (22).

All of the patients studied received a high caloric diet providing a quantity of vitamin A estimated (23) at approximately 14,000 I.U. per day in addition to adequate amounts of other vitamins and minerals. No obvious pathology of the eye was observed in any of the patients. None of the patients with Laennec's cirrhosis had jaundice or diarrhea at the time of the study. In most of these cases it was possible to determine the plasma vitamin A and carotenoid levels during completely afebrile periods.

The patients were divided for comparison into the following 3 clinical groups as judged by physical signs and laboratory tests: (1) patients with decompensated² Laennec's cirrhosis, (2) patients with compensated Laennec's cirrhosis, and (3) patients with certain other diseases (hospital controls). The results of the blood analyses and visual tests on these groups were compared with similar data previously obtained (20) in normal subjects.

The ratio of the concentration of plasma vitamin A to the concentration of plasma carotenoid (A/C ratio) was

² Patients with "decompensated" cirrhosis were bedridden. They generally gave the history of recent weight loss and failing strength. Examination showed a palpable liver and spleen, ascites, edema, and signs of collateral venous circulation. Laboratory tests gave evidence of severe derangement of liver function.

Patients with "compensated" cirrhosis were ambulatory. They felt well. Although the liver and spleen were usually palpable, there was no ascites, edema, or jaundice. Laboratory findings were less abnormal than in the decompensated group. The clinical differences between these groups are described more fully in another paper (19).

also compared among the 4 groups of subjects. If in Laennec's cirrhosis the *in vivo* conversion of carotene to vitamin A is disturbed, or if the levels of the two blood components are depressed to different extents by the disease process, this ratio should measure the degree of departure from normal relations.

The individual measurements are presented in Tables I to III, and the mean values, standard deviations, and ranges are given in Table IV. Also given in Table IV are similar data on normal adults elsewhere reported (20) and, in addition, unpublished plasma cholesterol values on the same normal subjects. Measurements in certain patients with Laennec's cirrhosis (numbers 4, 28, and 41) appear in both Tables I and II due to changes in their clinical condition.

In determining the statistical significance of the data,

TABLE I

Plasma vitamin A, carotenoid, and cholesterol, and dark adaptation time in patients with decompensated Laennec's cirrhosis

When the value recorded represents an average, the number of observations is indicated in parenthesis.

Patient	Sex	Associated diseases, etc.*	Plasma vitamin A	Plasma carotenoid	Vitamin A Carotenoid	Plasma cholesterol	Dark adaptation time
			<i>I.U. per cent</i>	<i>μgm. per cent</i>		<i>mgm. per cent</i>	<i>minutes</i>
58	f		5(2)	33(2)	0.15	168	17.5
7	m	A†	30	17	1.76		17.0(2)
5	m	A†	32	50	0.64	239	14.5
18	m	D	38	75	0.51	166	
60	m		41	45	0.91	242	
2	m	D	43(2)	60(2)	0.72	162	18.4
4	m		43(4)	79(4)	0.54	262(4)	23.9(5)
11	m	D	47(2)†	42(2)	1.12		36.0
36	m	D	47(3)	103(3)	0.46	262(3)	19.6
1	m	A (Carcinoma of liver)†	50	114	0.44		
37	f	D	56(3)	113(3)	0.50	143(3)	14.3
54	m	A†	58(3)	60(3)	0.97	212	18.7
38	m	A (Carcinoma of liver)	59	44	1.34	282	
15	f		61(7)	74(7)	0.82	230(5)	27.9(6)
12	f		62(3)	113(3)	0.55		20.5
35	m	D	67(2)	154(3)	0.44	204(3)	16.2
63	m		69	28	2.46		13.1
27	f	D	73	137	0.53		
14	m	Lues, B	75	69	1.09	185	13.2
59	f		77	36	2.14	352	21.0
17	f		82(2)†	64(2)	1.28	175	15.2
57	f		85	42	2.02	160	18.8
62	m		89	50	1.78	242	15.5
49	f		95	79	1.20	257	44.7
24	m		121	58	2.09	154	15.1
6	m	D	131(3)	166(3)	0.79	321(2)	14.3
19	m	A					16.0
23	f						25.7(2)
28	f	Acute glomerulonephritis					
		D					20.1(3)
41	m	A					18.5
42	m	B					19.7
43	f	A					23.1
44	m	A					17.6
46	m						14.9
47	f	B, D					18.7
48	m	D					16.0
50	f	A					19.0
51	m	D					24.0(5)
Mean			65	72	1.11	240	19.7

* A denotes autopsy, B biopsy, and D died, no autopsy.

† See Table V for vitamin A and carotenoid content of the liver.

‡ Fever may have contributed to the low value.

the methods and tables of R. A. Fisher (24) were employed. A difference between two mean values was considered significant if the probability of such a difference arising by chance was found to be less than 0.05 (1 chance in 20).

RESULTS

Decompensated cirrhosis. Table I shows individual measurements of 38 patients with decompensated Laennec's cirrhosis. These values may be compared with the range of normal values given in Table IV. In 24 of 26 cases the vitamin A level is below the lowest normal value; in 13 of 26 cases the carotenoid level is below the lowest normal value; in 26 of 33 cases the dark adaptation time is above the highest normal value; and in 8 of 26 cases the A/C ratio is below lowest normal and in no cases above highest normal.

Compensated cirrhosis. Table II shows individual measurements of 27 patients with compensated Laennec's cirrhosis. These are similar in most respects to those of Table I, but show less striking deviations from the normal. Moreover, in contrast to a tendency to low values shown by the decompensated cases, the A/C ratio is below normal in only 3 of 23 cases, while in 4 it is above normal. These quantitative differences between groups I and II imply that the degree of change in such measurements may afford a crude index to the degree of liver dysfunction.

Hospital controls. Table III presents data obtained from 38 patients having 25 miscellaneous diseases. In these patients a majority of the values are within the normal range. Of the abnormal values about one-half represent minimal deviations from the normal. It may be noted that the plasma cholesterol level is abnormally high in certain instances, as in the case of nephrosis. On this account the mean value for the entire group is above normal, although not significantly so (Table IV).

Table IV compares the ranges and mean values of the 3 groups of patients with those for corresponding measurements in normal persons. In calculating the mean dark adaptation time for the hospital controls there were included additional measurements of 40 patients having 16 miscellaneous diseases. Statistically significant differences exist between all the mean values in each column of Table IV except those designated by asterisks.

TABLE II

Plasma vitamin A, carotenoid, and cholesterol, and dark adaptation time in patients with compensated Laennec's cirrhosis

When the value recorded represents an average, the number of observations is indicated in parenthesis.

Patient	Sex	Associated diseases, etc.*	Plasma vitamin A <i>I.U. per cent</i>	Plasma carotenoid <i>μgm. per cent</i>	Vitamin A Carotenoid	Plasma cholesterol <i>mgm. per cent</i>	Dark adaptation time <i>minutes</i>
52	m	Myxedema	5(21)	170(21)	0.30	245(21)	27.3
8	m	A†§	24	179	0.13	207	
30	f		47	58	0.81	185	
13	m		72(3)	108(3)	0.67	195	11.3
16	f	Chronic glomerulo-nephritis	75(7)	247(3)	0.30	383(17)	18.7(4)
20	m		97†	108	0.90		18.5(2)
61	f		98	40	2.45	238	24.0
32	f	Polyneuritis	109	108	1.01	197	
29	f	Diabetes mellitus	115(2)	25(2)	4.60	556	14.4
28	m	D§	117	87	1.34		
3	m		113(12)	169(12)	0.79	271(8)	21.1(12)
26	m		141	58	2.43		
33	f	Polyneuritis	143(2)	79(2)	1.81	285	14.2
31	m	Polyneuritis	146(2)	31(2)	4.71	256(2)	
9	m		151(3)	122(3)	1.25	309(2)	16.2
4	m		152	48	3.17	312	
25	f	Polyneuritis	153(4)	51(4)	3.00	235(2)	
56	m		164	79	2.08	232	10.4
39	m		166	47	3.53	386	
41	m		167	8	20.90		
53	m		170	62	2.74	278	
34	f	Polyneuritis	175	28	6.25	250	13.8
10	m		181	112	1.62		16.2
21	m	Polyneuritis B					17.3
22	f	Polyneuritis					20.0
40	m						25.6
45	f						17.5
Mean			122	88	2.08	271	17.3

* A denotes autopsy, B biopsy, and D died, no autopsy.

† See Table V for vitamin A and carotenoid content of liver.

‡ Fever may have contributed to the low value.

§ Death not attributable to Laennec's cirrhosis.

|| Omitted in computing the mean. The cholesterol values were omitted because these patients had diseases known to induce abnormally high blood cholesterol levels.

The mean values (Table IV) of the plasma vitamin A and carotenoid, and of the dark adaptation time, grow progressively more abnormal in the following order of the groups: normal controls, hospital controls, compensated cirrhotics, and decompensated cirrhotics. Thus all three variables are influenced in the same direction by the presence and by the severity of Laennec's cirrhosis.

The most striking departure from normal and from the hospital controls is exhibited by the plasma vitamin A level of the decompensated cirrhotics (Table IV). The mean value of the plasma vitamin A for this group is only $\frac{1}{3}$ of normal compared with $\frac{1}{2}$ of normal for the carotenoid level and $1\frac{1}{2}$ times above normal for the dark adaptation time. Moreover, 92 per cent of

the vitamin A values are outside of the normal range compared with 50 per cent of the carotenoid, 31 per cent of the A/C ratio, and 79 per cent of the dark adaptation time values. The distribution of plasma vitamin A values within each of the 4 groups of subjects is shown graphically in Figure 1.

The plasma vitamin A level clearly differentiates the compensated from the decompensated group of cirrhotic patients, the mean vitamin A level of the latter group being only $\frac{1}{2}$ that of the former. Although the mean carotenoid level is lower and the mean dark adaptation time higher in the decompensated than in the compensated group, the differences are relatively small and lack statistical significance (Table IV).

The mean plasma A/C ratio of the decompensated

also compared among the 4 groups of subjects. If in Laennec's cirrhosis the *in vivo* conversion of carotene to vitamin A is disturbed, or if the levels of the two blood components are depressed to different extents by the disease process, this ratio should measure the degree of departure from normal relations.

The individual measurements are presented in Tables I to III, and the mean values, standard deviations, and ranges are given in Table IV. Also given in Table IV are similar data on normal adults elsewhere reported (20) and, in addition, unpublished plasma cholesterol values on the same normal subjects. Measurements in certain patients with Laennec's cirrhosis (numbers 4, 28, and 41) appear in both Tables I and II due to changes in their clinical condition.

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25	f	Polyneuritis	153(4)	51(4)	3.00	235(2)	
56	m		164	79	2.08	232	10.4
39	m		166	47	3.53	386	
41	m		167	8	20.90		
53	m		170	62	2.74	278	
34	f	Polyneuritis	175	28	6.25	250	13.8
10	m		181	112	1.62		16.2
21	m	Polyneuritis B					17.3
22	f	Polyneuritis					20.0
40	m						25.6
45	f						17.5
Mean			122	88	2.08	271	17.3

* A denotes autopsy, B biopsy, and D died, no autopsy.

† See Table V for vitamin A and carotenoid content of liver.

‡ Fever may have contributed to the low value.

§ Death not attributable to Laennec's cirrhosis.

|| Omitted in computing the mean. The cholesterol values were omitted because these patients had diseases known to induce abnormally high blood cholesterol levels.

The mean values (Table IV) of the plasma vitamin A and carotenoid, and of the dark adaptation time, grow progressively more abnormal in the following order of the groups: normal controls, hospital controls, compensated cirrhotics, and decompensated cirrhotics. Thus all three variables are influenced in the same direction by the presence and by the severity of Laennec's cirrhosis.

The most striking departure from normal and from the hospital controls is exhibited by the plasma vitamin A level of the decompensated cirrhotics (Table IV). The mean value of the plasma vitamin A for this group is only $\frac{1}{2}$ of normal compared with $\frac{1}{2}$ of normal for the carotenoid level and $1\frac{1}{2}$ times above normal for the dark adaptation time. Moreover, 92 per cent of

the vitamin A values are outside of the normal range compared with 50 per cent of the carotenoid, 31 per cent of the A/C ratio, and 79 per cent of the dark adaptation time values. The distribution of plasma vitamin A values within each of the 4 groups of subjects is shown graphically in Figure 1.

The plasma vitamin A level clearly differentiates the compensated from the decompensated group of cirrhotic patients, the mean vitamin A level of the latter group being only $\frac{1}{2}$ that of the former. Although the mean carotenoid level is lower and the mean dark adaptation time higher in the decompensated than in the compensated group, the differences are relatively small and lack statistical significance (Table IV).

The mean plasma A/C ratio of the decompen-

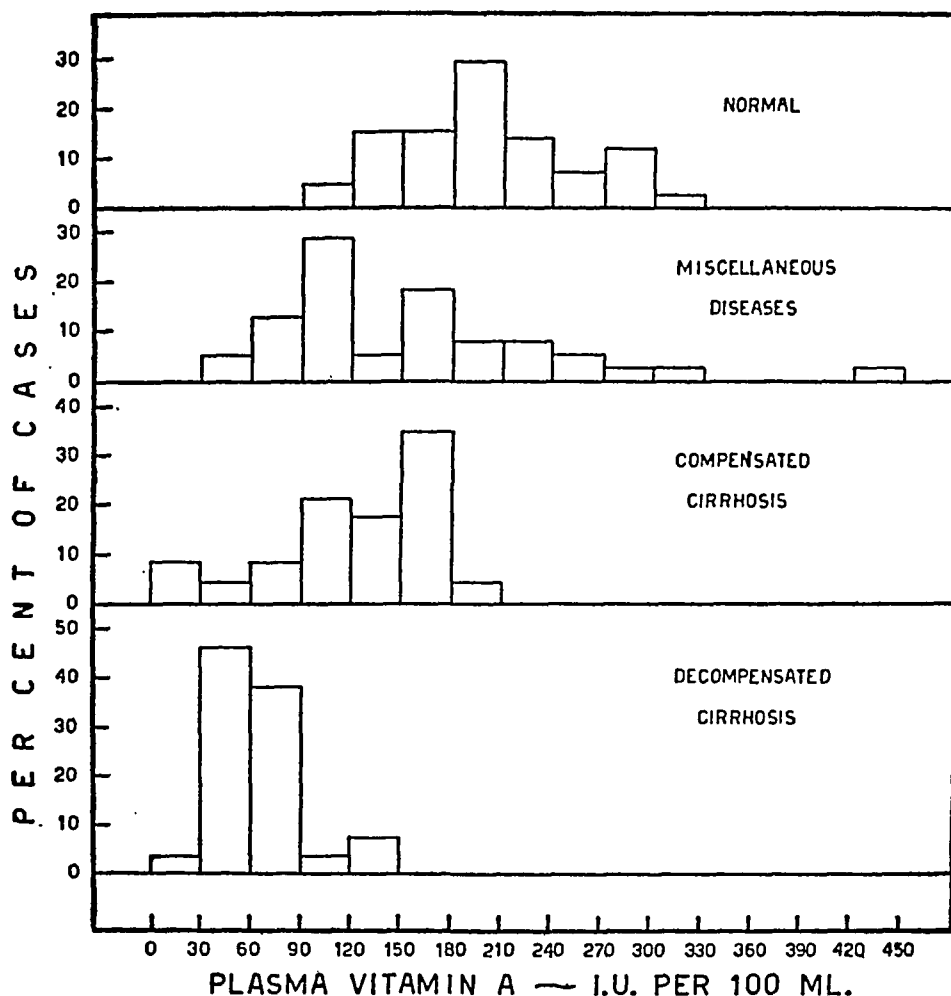


FIG. 1. PERCENTAGE DISTRIBUTION OF PLASMA VITAMIN A VALUES IN CLASS INTERVALS OF 30 I.U. PER CENT FOR THE 4 GROUPS OF SUBJECTS

plasma vitamin A values were well within the normal limits, being respectively 118, 170, 295, and 202 I.U. per cent.

Vitamin A and carotenoid content of cirrhotic livers. Table V presents the vitamin A and carotenoid concentrations found in 6 cirrhotic livers at autopsy, 5 being from patients in the present series. The mean vitamin A concentration of 124 I.U. per gram, carotenoid concentration of 4.3 μ gm. per gram, and A/C ratio of 36 are in close agreement with corresponding values reported by Ralli *et al.* (7) for the livers of 15 persons who died of cirrhosis of the liver. These workers found mean values of 137 I.U. per gram for vitamin A, 5.7 μ gm. per gram for carotenoid, and 31 for the A/C ratio. Their findings for 23 normal persons killed in accidents may therefore be compared with the present values obtained in cirrhotic livers. The mean values for these normal livers were 766 I.U. per gram for vitamin A,

13.6 μ gm. per gram for carotenoid, and 74 for the A/C ratio (7).

DISCUSSION

It is significant that of the 12 patients with compensated cirrhosis for which there are visual as well as blood measurements, 3 have abnormal dark adaptation times although the concentrations of the blood components are normal. While two of the abnormal dark adaptation values are not particularly high (16.2 minutes), one is strikingly abnormal (21.1 minutes). To understand this phenomenon it is necessary to recall the evidence (3, 4, 20, 27, 28) that abnormal dark adaptation of the type encountered in Laennec's cirrhosis, *i.e.*, delayed, with or without elevation of the final threshold, is an index of faulty utilization of vitamin A by the retina. This delayed type of adaptation, involving a disturbance in the kinetics of the retinal process (presumably a slowing of

the rate of visual purple regeneration), is to be distinguished from the simple elevation of the visual threshold observed in experimental vitamin A deficiency (12). Apparently, the liver normally contributes to the functioning of the visual mechanism independently of its rôle as the principal vitamin A depot. Thus an abnormally high dark adaptation time in a patient with Laennec's cirrhosis may be entirely consistent with concomitant normal vitamin A and carotenoid levels.

Of more frequent occurrence among patients with cirrhosis is an abnormal plasma vitamin A or carotenoid level with a normal dark adaptation time, *viz.*, 5 of 21 decompensated and 3 of 12 compensated cases. This indicates the presence in these patients of vitamin A deficiency *per se* which is presumably not sufficiently severe or prolonged to raise the dark adaptation time to an abnormal level. It likewise shows the absence in these subjects of the faulty utilization of vitamin A by the retina, characteristic of Laennec's cirrhosis (3, 4).

Although these patients did not have jaundice or diarrhea at the time of observation, it is possible that the low values for plasma vitamin A are related in part to malabsorption from the intestinal tract. It seems improbable that this is the only factor involved, since there is a correlation between the degree of vitamin A deficiency and the apparent degree of liver incompetence. Previous studies (3, 4) on dark adaptation in patients with Laennec's cirrhosis also showed changes that were not explicable on the basis of malabsorption alone.

In addition to those mentioned, there are a number of other factors known to exert independent effects upon the plasma vitamin A level or the dark adaptation function: alcohol mobilizes vitamin A in the blood (30) and elevates the visual threshold (31); febrile conditions, as well as reduced vitamin A intake, may depress the blood vitamin A level without measurably influencing the visual threshold (17, 18, 29); thyroid extract and α -dinitrophenol, in certain conditions, simultaneously increase the speed and extent of dark adaptation and depress the blood level of vitamin A (27), glucose lowers the visual threshold when the latter is elevated by anoxemia (32); retinitis pigmentosa and optic nerve atrophy are

TABLE V
Concentration of vitamin A and carotenoid in cirrhotic livers

Patient	Vitamin A	Carotenoid	Vitamin A Carotenoid
	I.U. per gram	μ m. per gram	
1*	305	3	102
5	35	2	18
7	121	6	20
8	161	10	16
54	102	3	51
64	21	2	11
Mean	124	4.3	36
Standard deviation	121	3.2	35

* Primary cancer of liver present.

associated with high visual thresholds (31), although neither condition is attributable to vitamin A deficiency. Thus the relation between vitamin A supply and visual sensation is complicated by numerous remote as well as proximal factors whose contributions must be estimated for a precise evaluation of the end result.

The present evidence renders it highly probable that the abnormal values of the vitamin A and carotenoid levels in the blood and liver, and of the dark adaptation time, observed in patients with Laennec's cirrhosis, are attributable primarily to the presence of cirrhosis *per se* rather than to any combination of the several extraneous factors enumerated above.

SUMMARY AND CONCLUSIONS

1. The dark adaptation time and the concentration of vitamin A and of total carotenoid in the blood plasma were determined in 49 patients with Laennec's cirrhosis and in 38 patients with various other diseases. The vitamin A and carotenoid concentrations were also determined in 6 cirrhotic livers. The vitamin A intake was controlled and the cirrhotic patients were free of jaundice, of diarrhea and, in most instances, of fever.

2. Compared with the mean normal level of 198 international units of vitamin A per 100 ml. of plasma, the mean values for the patients were as follows: decompensated cirrhosis, 65 I.U. per cent; compensated cirrhosis, 122 I.U. per cent; miscellaneous diseases, 154 I.U. per cent. Ninety-two per cent of the patients with decompensated cirrhosis had values below the lowest normal.

3. Compared with the mean normal level of 144 micrograms of total carotenoid per 100 ml. of plasma, the mean values for the patients were as follows: decompensated cirrhosis, 72 μ gm. per cent; compensated cirrhosis, 88 μ gm. per cent; miscellaneous diseases, 121 μ gm. per cent. Fifty per cent of the patients with decompensated cirrhosis had values below the lowest normal.

4. Compared with the mean normal dark adaptation time of 13.1 minutes, the mean values for the patients were as follows: decompensated cirrhosis, 19.7 minutes; compensated cirrhosis, 17.3 minutes; miscellaneous diseases, 15.2 minutes. Seventy-nine per cent of the patients with decompensated cirrhosis had values above the highest normal.

5. Compared with the mean normal concentrations of 766 I.U. per gram of vitamin A and 13.6 μ gm. per gram of carotenoid, 6 cirrhotic livers had concentrations of 124 I.U. per gram of vitamin A and 4.3 μ gm. per gram of carotenoid.

6. It is concluded that (1) the incidence and the degree of vitamin A deficiency, as measured by the level of vitamin A and of carotenoid in the blood and liver, and by the dark adaptation time, are greater in Laennec's cirrhosis than in the mixed group of diseases used as controls in the present study; (2) among these three variables the one most markedly influenced by the presence and by the severity of Laennec's cirrhosis is the blood vitamin A level, which appears to be a crude index of the severity of the disease process.

BIBLIOGRAPHY

1. Breusch, F., and Scalabrino, R., Die quantitativen Verhältnisse der Leberlipoide. *Ztschr. f. d. ges. exper. Med.*, 1934, 94, 569.
2. Lasch, F., Über den Vitamin A—Spiegel im Blute bei Leberkrankheiten. *Klin. Wchnschr.*, 1938, 17, 1107.
3. Haig, C., Hecht, S., and Patek, A. J., Jr., Vitamin A and rod-cone dark adaptation in cirrhosis of the liver. *Science*, 1938, 87, 534.
4. Patek, A. J., Jr., and Haig, C., The occurrence of abnormal dark adaptation and its relation to vitamin A metabolism in patients with cirrhosis of the liver. *J. Clin. Invest.*, 1939, 18, 609.
5. Rubegni, R., Il contenuto in vitamin A e in carotina del siero umano in varie condizioni patologiche. *Policlinico (sez. med.)*, Rome, 1939, 46, 565.
6. Woo, T. T., and Chu, F. T., Vitamin A content of livers of chinese infants, children and adults. *Chinese J. Physiol.*, 1940, 15, 83.
7. Ralli, E. P., Papper, E., Paley, K., and Baumann, E., Vitamin A and carotene content of human liver in normal and in diseased subjects: an analysis of one hundred and sixteen human livers. *Arch. Int. Med.*, 1941, 68, 102.
8. Bajardi, G., and Galeone, A., La cecità notturna quale sintomo di malattia epatica. *Policlinico (sez. prat.)*, 1941, 48, 193.
9. Ralli, E. P., Baumann, E., and Roberts, L. B., The plasma levels of vitamin A after ingestion of standard doses: studies in normal subjects and patients with cirrhosis of the liver. *J. Clin. Invest.*, 1941, 20, 709.
10. McCoord, A. B., and Luce-Clausen, E. M., The storage of vitamin A in the liver of the rat. *J. Nutrition*, 1934, 7, 557.
11. Lewis, J. M., and Haig, C., Vitamin A requirements in infancy as determined by dark adaptation. *J. Pediat.*, 1939, 15, 812.
12. Hecht, S., and Mandelbaum, J., Dark adaptation and experimental human vitamin A deficiency. *Am. J. Physiol.*, 1940, 130, 651.
13. Lewis, J. M., Bodansky, O., Falk, K. G., and McGuire, G., Relationship of vitamin A blood level in the rat to vitamin A intake and to liver storage. *Proc. Soc. Exper. Biol. and Med.*, 1941, 46, 248.
14. Horton, P. B., Murrill, W. A., and Curtis, A. C., Vitamin A and carotene. I. The determination of vitamin A in the blood and liver as an index of vitamin A nutrition of the rat. *J. Clin. Invest.*, 1941, 20, 387.
15. Crimm, P. D., and Short, D. M., Vitamin A content of human liver in tuberculosis. *Ann. Int. Med.*, 1939, 13, 61.
16. Breese, B. B., and McCoord, A. B., Vitamin A absorption in catarrhal jaundice. *J. Pediat.*, 1940, 16, 139.
17. Clausen, S. W., and McCoord, A. B., The carotenoids and vitamin A of the blood. *J. Pediat.*, 1938, 13, 635.
18. Lewis, J. M., Bodansky, O., and Haig, C., Level of vitamin A in the blood as an index of vitamin A deficiency in infants and in children. *Am. J. Dis. Child.*, 1941, 62, 1129.
19. Patek, A. J., Jr., and Post, J., Treatment of the liver by a nutritious diet and supplements rich in vitamin B complex. *J. Clin. Invest.*, 1941, 20, 481.
20. Haig, C., and Patek, A. J., Jr., The relation between dark adaptation and the level of vitamin A in the blood. (To be published.)
21. Haig, C., and Post, J., Vitamin A concentration in rat liver during recovery from CCl₄ cirrhosis. *Proc. Soc. Exper. Biol. and Med.*, 1941, 48, 710.
22. Bloor, W. R., Pelkin, K. F., and Allen, D. M., Determination of fatty acids (and cholesterol) in small amounts of blood plasma. *J. Biol. Chem.*, 1922, 52, 191.

23. Daniel, E. P., and Munsell, H. E., Vitamin content of foods. U. S. Dept. of Agric. misc. public. 275, June, 1937.
24. Rider, P. R., An Introduction to Modern Statistical Methods. John Wiley & Sons, New York, 1939.
25. Josephs, H. W., Studies in vitamin A; relation of vitamin A and carotene to serum lipids. Bull. Johns Hopkins Hosp., 1939, 65, 112.
26. Post, J., and Patek, A. J., Jr., Serum proteins in cirrhosis of the liver. I. Their relation to prognosis and to the formation of ascites. Arch. Int. Med., 1942, 69, 67.
27. Haig, C., Vitamin A and the rates of adaptation of the eye to light and darkness. Anat. Rec., 1940, 78, suppl., 163.
28. Patek, A. J., Jr., and Haig, C., Effect of administration of thyroid extract and of α -dinitrophenol upon dark adaptation. Proc. Soc. Exper. Biol. and Med., 1941, 46, 180.
29. Bodansky, O., Lewis, J. M., and Haig, C., The comparative value of the blood plasma vitamin A concentration and the dark adaptation as a criterion of vitamin A deficiency. Science, 1941, 94, 370.
30. Clausen, S. W., Baum, W. S., McCoord, A. B., Rydeen, J. A., and Breese, B. B., Mobilization of vitamin A from its stores in the tissues by ethyl alcohol. Science, 1940, 91, 318.
31. Mandelbaum, J., Dark adaptation. Some physiologic and clinical considerations. Arch. Ophth., New York, 1941, 26, 203.
32. McFarland, R. A., and Evans, J. M., The effects of variations in the concentration of oxygen and of glucose on dark adaptation. J. Gen. Physiol., 1940, 24, 69.

THE EFFECT OF EXTERNAL PRESSURE ON THE VASCULAR VOLUME OF THE FOREARM AND ITS RELATION TO CAPILLARY BLOOD PRESSURE AND VENOUS PRESSURE

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While the direct measurement of capillary blood pressure in man by means of a micro-pipette (1, 2, 3) is quite accurate, this method has several disadvantages which have prevented wide application to clinical problems. They are (a) the great variability of readings in single capillaries, which makes it practically impossible to obtain a statistically significant number of readings in a given patient at any one time in his illness; (b) the restriction of this method to the capillaries of the skin of the nail fold where the vessels may not be typical of those in muscle or general connective tissue; and (c) the nearness of arterio-venous anastomoses which, by opening and closing, may disturb pressures in the nearby capillaries of the nail fold.

The indirect methods are easily applied to clinical problems but, as pointed out previously (4), lead to widely different estimates of blood pressure in the capillaries or minute vessels as a whole, as might be expected from the subjective and arbitrary nature of the criteria adopted by each investigator to indicate a state of balance between the externally applied pressure and capillary blood pressure. Eichna and Bordley (3) have compared the direct and indirect procedures; they concluded that the usual capsular method, with microscopic observation of the collapse of individual capillaries, was wholly undependable.

Fundamentally, nearly all indirect methods for measuring blood pressure in the minute vessels involve the application of external pressure to diminish the volume of one or more capillaries, using visual evidence such as cessation of flow, modification of flow, or changes in skin color to indicate when the applied pressure is great enough to obliterate or at least flatten, the capillaries under observation. It seems possible, however,

that the average pressure in the minute vessels of human skin and muscle might be estimated plethysmographically by determining the effect of graded external pressure on the collective vascular volume of the forearm. The plethysmograph, by adding together the infinitesimal changes in volume of many individual vessels, might then provide an average and more objective measure of blood pressure in the capillaries, or at least in the minute vessels as a whole.

This paper describes a series of observations designed to determine (a) the effect of graded external pressure on the volume of blood in the human forearm; (b) the relation of these pressure-volume curves to the previously reported direct readings of capillary blood pressure and venous pressure under like conditions; and (c) the effect of known changes of capillary blood pressure on the pressure-volume curves, for the purpose of assessing the validity and usefulness of this procedure as a method of estimating blood pressure in the minute vessels of man during disease.

APPARATUS

Whereas the usual form of plethysmograph measures changes in arm volume at or very near atmospheric pressure, Landis and Gibbon (5) devised a "pressure plethysmograph" to measure limb volume under external pressures as high as 200 mm. Hg. In previous studies (5) high external pressure was used in order to collapse the blood vessels completely and thus permit accurate estimation of the volume of extravascular fluid. Conversely, in the studies now reported, the pressure applied to the forearm in the plethysmograph ranged from 5 to 120 mm. Hg, while circulation was stopped temporarily, and then restored, to measure the effect of graded external pressure on vascular volume.

The plethysmograph itself required no important modification from that described by Landis and Gibbon (5) and diagrammed on page 110 of that paper, to which the reader is referred for details. The accessory portions of

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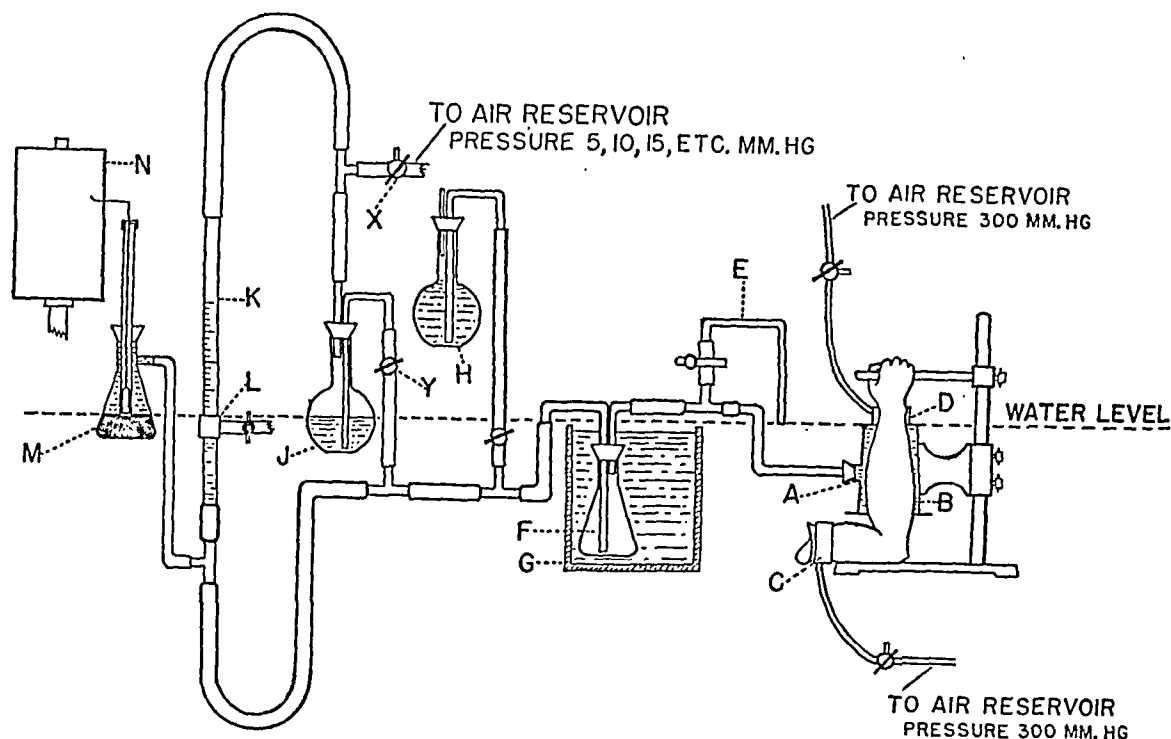


FIG. 1. DIAGRAM OF PRESSURE PLETHYSMOGRAPH AND ATTACHED APPARATUS
For description see text. For details of pressure plethysmograph see p. 110 of reference 5.

the apparatus required several changes. The large opening into the center of the plethysmograph (Figure 1, A) was closed by a rubber stopper containing a short piece of glass tubing with an inside diameter of 10 mm. This was connected by heavy-walled pressure tubing of 9 mm. bore to a suitable burette (Figure 1, K). The lower end of the burette was cut off so that it could be inserted directly into the larger rubber tubing which, with large T-tubes, was used to facilitate rapid movement of water throughout the system. A water outlet (Figure 1, E) was adjusted to the height of the plethysmograph, to permit accurate equalization of fluid levels within the system at the beginning of each observation. A 1000 ml. flask (Figure 1, F) was immersed in a 20-litre water bath, kept at constant temperature (Figure 1, G), and between the burette and plethysmograph so that as water at room temperature was expelled from the burette into the lower portion of the flask, an equal volume of water at the desired temperature passed from the top of the flask into the plethysmograph, thus maintaining the temperature of the plethysmograph and forearm at the desired level. A 1500 ml. flask (Figure 1, H), with an airhole in its stopper, was used merely as a reservoir to introduce or withdraw water from the system in preliminary adjustment and at the end of an experiment.

For measuring changes in volume two different burette systems were found necessary. In some experiments, chiefly observations 1, 2 and 6, below, where large or gross changes in volume were measured, a 250 cc. dispensing burette was used alone (Figure 1, K; omitting J and Y). This burette, partially filled with water, communicated by its lower end with the water in the interior of the plethysmograph, and by its upper end, through a

three-way stopcock (Figure 1, X), with a 40-liter reservoir (not shown), which contained air previously brought to the desired pressure as indicated by a separate mercury manometer (not shown). Through the three-way stopcock (X) pressure could be applied suddenly to water in the burette and thence to the forearm, where changes in total volume were followed by observing the reading opposite the water meniscus in the burette. To maintain the pressure on the forearm constant, it was of course necessary to move the burette continuously in the holder, L, so that the water meniscus remained opposite the line marked "water level" in Figure 1.

In other observations much smaller changes, comprising vascular volume only, had to be measured more accurately. For these, a 50 cc. Shellbach burette (Figure 1, K) with graduations of 0.1 cc. was used. To suppress the bulk of the preliminary physical change in total volume, which accompanied each change of plethysmograph pressure, a 1000 cc. flask (Figure 1, J) half filled with water, and a stopcock (Figure 1, Y) were inserted in parallel to the burette. As in the burette, the water in the lower part of the flask communicated with the water in the plethysmograph while the air in the upper part of the flask communicated through the three-way stopcock (X) with the air reservoir. With stopcock Y open, any pressure applied through stopcock X displaced fluid from the flask while the volume of water in the burette, except for transient fluctuation, remained almost constant. As the gross shift of fluid ceased, stopcock Y was closed and thereafter smaller changes in volume were followed more accurately by observing the movement of water in the small burette. Whichever burette was used, a mercury manometer (Figure 1, M) recorded on a kymo-

graph (Figure 1, N) the level and constancy of the pressure exerted by the plethysmograph on the forearm.

Two pneumatic cuffs (Figure 1, C and D), 44 cm. long and 5.5 cm. wide, were available for occluding circulation just below the axilla and at the wrist, respectively. These were connected to another 40-liter reservoir (not shown) containing air under a pressure of 300 mm. Hg. The wrist cuff was kept inflated throughout each observation to exclude possible errors arising from the return of blood from the hand to the veins of the forearm within the plethysmograph.

The temperature of the forearm and surrounding water was controlled by pumping fluid from a water bath (Figure 1, G) through the double-walled jacket of the plethysmograph. A bimetallic thermoregulator kept the water in the bath constantly at the desired temperature, which in all the following observations was 34° C.

GENERAL METHODS

The effect of graded external pressure on vascular volume was measured in several ways. Since the initial preparations for all methods were similar they will be described first. An illustrative protocol is given at the end of the paper.

The plethysmograph-burette system, having been brought to a temperature of 34° C., was prepared by removing all air bubbles, leaving enough water in the system to keep the burette and flasks suitably filled and the thin rubber sleeve collapsed against the inner wall of the plethysmograph. The redundant portions of the collapsed plethysmograph sleeve were arranged in folds equally at the two ends of the plethysmograph and the washers, aluminum plates, and brass rings were assembled loosely in their proper positions.

With the subject supine, a coating of talc was applied to the skin of the forearm so that the rubber sleeve (Figure 1, B) might move smoothly over the skin. The hand was slipped into the plethysmograph, which was then lowered over the forearm until its midpoint lay at the level of the manubrium sterni. In its final position the upper arm was abducted about 45°; the elbow was flexed at right angles to the upper arm so that the forearm was perpendicular to the table. The elbow and distal quarter of the posterior surface of the arm rested on the base of the iron standard (see Figure 1) to prevent the conical forearm from being pushed out of the plethysmograph when pressure was applied. The fingers were flexed lightly over a horizontal rod containing a wood hand grip and covered loosely with adhesive tape. The inner light rubber portions of the washers, along with the redundant sleeve ends, were invaginated along the forearm, and the aluminum diaphragms were clamped tightly in position with their edges near, but not actually touching, the skin. Special care was necessary in this adjustment to prevent subsequent tightness of the diaphragms from producing venous congestion.

The overflow tube (Figure 1, E) was adjusted so that its orifice was level with the upper (wrist) diaphragm of the plethysmograph. The water in flask J, the marker

for the burette (Figure 1, L), and the mercury manometer (Figure 1, M) were all brought accurately to this same level.

One of the narrow pneumatic cuffs was wrapped around the wrist immediately above the upper aluminum diaphragms, and the second cuff was placed as high as possible on the upper arm.

Stopcock X was opened to room air to permit water to enter the burette and reservoir J was isolated from the system temporarily to avoid changing its water level during the filling period. The burette-plethysmograph system was filled from flask H with enough water to produce a slight positive pressure in the plethysmograph and thus to force the rubber sleeve closely against the forearm. The pressure then was reduced to atmospheric level by opening the overflow tube E and simultaneously stopcock Y was opened to allow water in the burette reservoir to assume precisely the level of the water in the burette and plethysmograph.

After closing the overflow tube E, the pressure in the plethysmograph was raised to 100 mm. Hg and lowered again to zero in a few seconds; this was repeated three times in quick succession to settle the rubber sleeve in position. Thereafter the water meniscus in the burette was kept opposite the point of reference (L) by moving the burette either up or down in its holder. Since the volume and pressure of water *per se* remained the same throughout each observation, changes in the volume of the forearm could be determined directly from the burette readings, while pressure in the plethysmograph was indicated simultaneously by the mercury manometer (M). From this point on, methods varied considerably and will be presented in the form of descriptions or specific protocols accompanying the corresponding observations.

OBSERVATIONS

1. *The pressure-volume curve of the burette and plethysmograph system alone*

The application of pressure to the interior of a system which is not absolutely rigid, because of rubber tubing and slight bulging of the diaphragms, will mechanically increase the volume of the entire system. To determine the magnitude of these changes, and the time required for a purely physical equilibrium to develop at each pressure, a glass cylinder (to represent the forearm) was placed in the plethysmograph and pressure was raised stepwise from 0 to 120 mm. Hg. Curve A in Figure 2 illustrates the magnitude of the observed changes in volume. At a pressure of 10 mm. Hg the volume of the system increased by approximately 13 cc.; at 20 mm. Hg by 20 cc.; and at 120 mm. Hg by 39 cc. For any one setting of the plethysmograph and its diaphragms

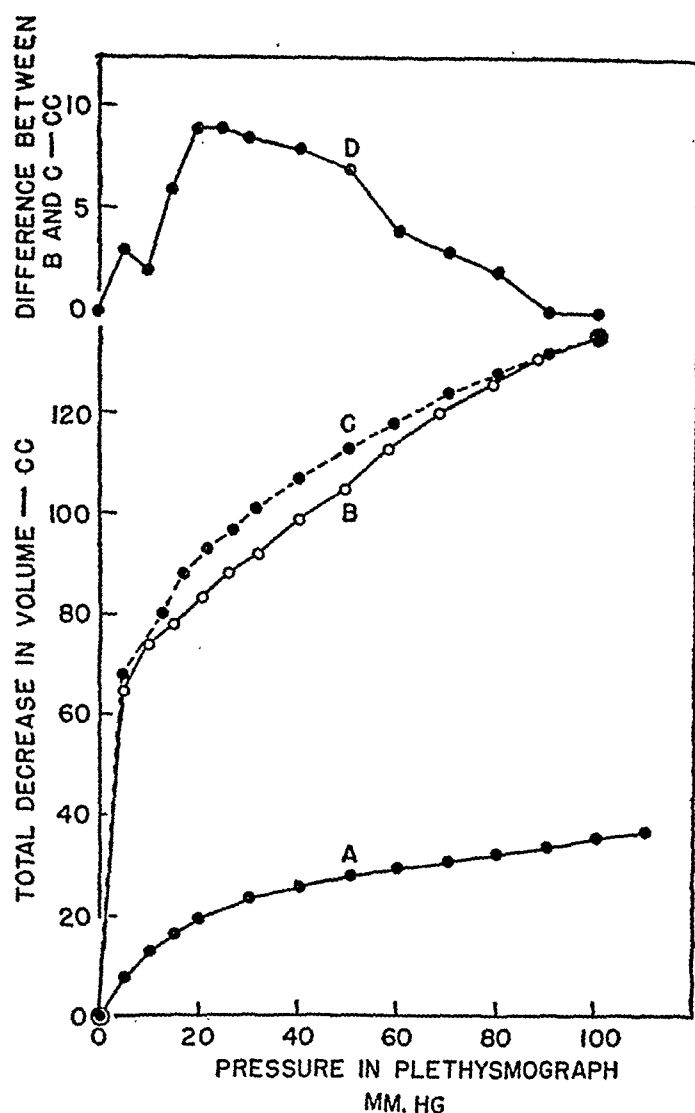


FIG. 2. SHOWING EFFECT OF PRESSURE ON VOLUME

Of burette-plethysmograph system containing glass cylinder (curve A); of forearm with circulation free (curve B); and of forearm with circulation occluded at the axilla (curve C). Curve D represents the difference between curve C and B, or "dynamic vascular volume."

the shape and position of rapidly repeated curves were extremely constant, indicating uniform and dependable apposition of the thin rubber sleeve against the cylinder within the plethysmograph, as well as uniform distortion of the diaphragms. After each change of pressure it required less than 3 seconds to reach physical equilibrium.

When high pressures were applied for several hours, slight but definite irreversible stretching of the system was observed. Thus at intervals of 30 minutes, in one observation a pressure of 120 mm. Hg increased the volume of the system by 37.0, 37.0, 37.0, 37.5 and 39.5 cc. At lower pressures, between 5 and 40 mm. Hg, this effect was also

present but considerably less, amounting to no more than 1.0 cc. over a 3-hour period.

These observations made it obvious that until an absolutely indistensible plethysmograph system can be devised, studies of pressure-volume relationship must be arranged (a) to exclude, or at least to cancel out, errors arising from expansion of the plethysmograph and its tubing; and, (b) to eliminate the slight but definite distension accompanying prolonged internal pressure by making each separate observation as brief as possible.

2. Gross pressure-volume curves of the forearm (a) with circulation intact and (b) with circulation occluded

When the forearm was placed in the plethysmograph the position and shape of the gross pressure-volume curve depended upon the presence or absence of circulation as shown in Figure 2, curves B and C respectively. In this observation the forearm of a normal male subject was placed in the plethysmograph as described under "General Methods," using the 250 cc. burette. The temperature of the forearm and plethysmograph was 34° C., while room temperature was 29° C. One narrow pneumatic cuff was wrapped around the wrist just distal to the plethysmograph and another as high as possible on the upper arm just distal to the axilla.

At zero time the wrist cuff was inflated to 30 mm. Hg to exclude the flow of blood to and from the hand. At the end of one minute a burette reading was recorded and from it, as a zero point, succeeding changes in volume were computed. Pressure was first raised to 5 mm. Hg and a relatively large volume of water moved rapidly from the burette into the plethysmograph. Since equilibrium with the forearm was reached more slowly than with the glass cylinder, one minute was allowed to elapse before reading the gross change in volume corresponding to an external pressure of 5 mm. This volume having been recorded as 65 cc., pressure was raised to 10 mm. Hg, at which pressure the volume 74 cc. was reached after one minute, and so on by steps until a pressure of 100 mm. Hg was attained as shown in the completed curve B of Figure 2. The pressure within the plethysmograph was then reduced to zero and the wrist cuff was deflated.

Since the observation required 12 minutes, a rest period of like duration was allowed so that reactive hyperemia might subside completely. The subject remained as quiet as possible to prevent any shift in the relative position of the forearm and plethysmograph. At the end of the rest period, with a total elapsed time of 24 minutes, the wrist cuff and also the cuff on the upper arm were inflated to 300 mm. Hg, thus occluding circulation to and from the forearm, except for such small amounts of blood as might pass through anastomotic connections within bone.

A zero reading at zero pressure was again recorded, differing from the first by 2 cc. which indicated a slight but definite shift in the position of the forearm relative to the plethysmograph. From this new zero, curve C of Figure 2 was determined in the manner already described and represented therefore the gross pressure-volume curve of the forearm with circulation occluded.

This latter curve C includes the instrumental changes in volume shown in curve A plus additional mechanical, or non-vascular changes in volume due to the shape and consistency of the forearm. Owing to its conical shape, the forearm tends to move outward from the larger end of the plethysmograph as pressure is raised. This movement is largely, but not completely, prevented by the heavy iron base against which the soft tissues of the elbow rest. Moreover, the plastic tissues of the forearm are to some extent pushed through the openings of the diaphragms as pressure increases.

Curve B includes these mechanical effects plus the distortion produced by active circulation. When blood flow is present and when intravascular pressure is being maintained the forearm resists certain external pressures, notably from 20 to 35 mm. Hg, more than others. This causes curve B to deviate from curve C slightly at low pressures, maximally at pressures between 20 and 35 mm. Hg and again less at pressures of 40 mm. Hg or more. Subtracting curve B from curve C produces curve D (Figure 2) which represents the effect of the *vis a tergo* of blood flow (and intravascular pressure) upon the gross pressure-volume curve, or the effect of external pressure upon "dynamic vascular volume" of the forearm, using that term because the conditions

under which the original curves B and C were determined differ chiefly in that arterial inflow was present in one instance (B) and occluded in the other (C). The relation between "dynamic vascular volume" and total vascular volume will be discussed in greater detail below. It is sufficient here to point out that "dynamic vascular volume" was greatest when external pressure was between 20 and 35 mm. Hg, and became less when external pressure was above or below this range.

This relationship held qualitatively when the two curves were determined in reverse order and also when pressure was started at 100 mm. Hg with stepwise reduction to zero. From the quantitative standpoint, however, the results shown in Figure 2 are distinctly better than average. Numerous trials indicated that this method was not adequate for accurate quantitative studies. Slight movement of the arm during the half hour or more required for a complete determination distorted the relation between the two curves and required transpositions which made the results less objective and accurate. The large total volumes involved made it necessary to use a 250 cc. burette with calibrations of 1 cc. whereas the "dynamic vascular volumes" being measured ranged from 3 to 20 cc. For these reasons the method was modified to measure "dynamic vascular volume" only, excluding changes in volume due to mechanical effects. The above observations have been included merely to indicate by the simplest possible method that graded external pressure produces disproportionate changes in the vascular volume of the forearm, and that the summit of this curve is associated with external pressures having an order of magnitude similar to that of capillary blood pressure.

3. Measurement of "dynamic vascular volume" alone at 34° C.

Three methods were used, all of which excluded from measurement the preliminary mechanical changes of volume due to the effects of pressure on the burette-plethysmograph system and on the non-vascular portions of the forearm. The 50 cc. burette, calibrated to 0.1 cc., and the reservoir were inserted in place of the 250 cc. burette.

Methods I and II. In Method I external pressures ranging from 0 to 70 mm. Hg were applied to the surface

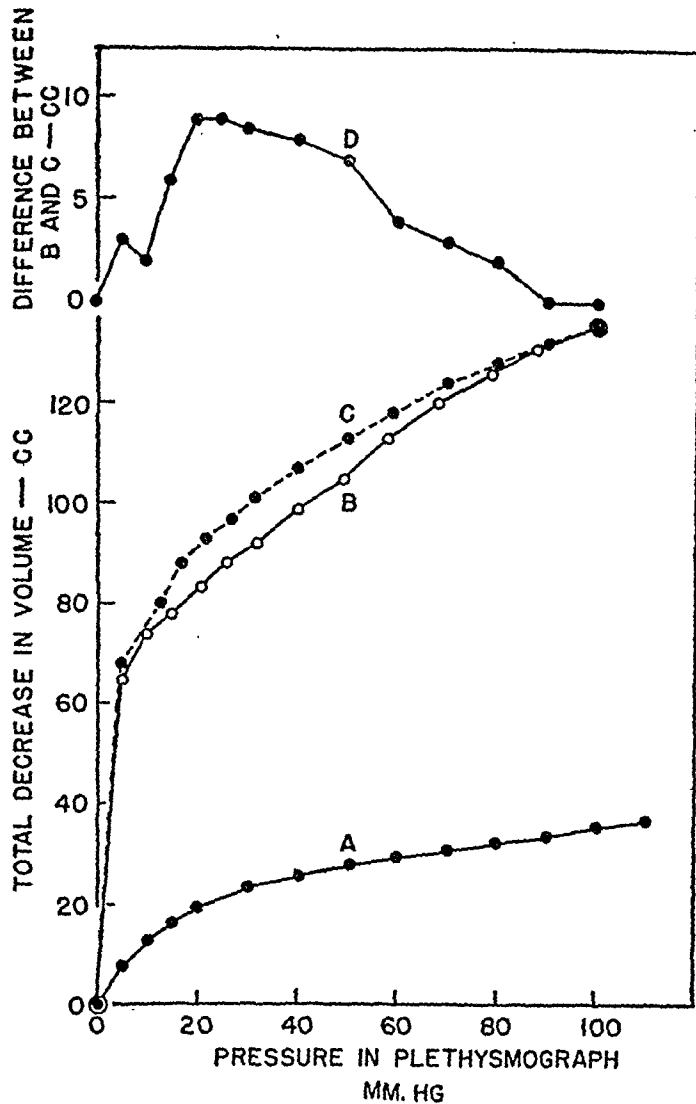


FIG. 2. SHOWING EFFECT OF PRESSURE ON VOLUME

Of burette-plethysmograph system containing glass cylinder (curve A); of forearm with circulation free (curve B); and of forearm with circulation occluded at the axilla (curve C). Curve D represents the difference between curve C and B, or "dynamic vascular volume."

the shape and position of rapidly repeated curves were extremely constant, indicating uniform and dependable apposition of the thin rubber sleeve against the cylinder within the plethysmograph, as well as uniform distortion of the diaphragms. After each change of pressure it required less than 3 seconds to reach physical equilibrium.

When high pressures were applied for several hours, slight but definite irreversible stretching of the system was observed. Thus at intervals of 30 minutes, in one observation a pressure of 120 mm. Hg increased the volume of the system by 37.0, 37.0, 37.0, 37.5 and 39.5 cc. At lower pressures, between 5 and 40 mm. Hg, this effect was also

present but considerably less, amounting to not more than 1.0 cc. over a 3-hour period.

These observations made it obvious that until an absolutely indistensible plethysmograph system can be devised, studies of pressure-volume relationship must be arranged (a) to exclude, or at least to cancel out, errors arising from expansion of the plethysmograph and its tubing; and, (b) to eliminate the slight but definite distension accompanying prolonged internal pressure by making each separate observation as brief as possible.

2. Gross pressure-volume curves of the forearm (a) with circulation intact and (b) with circulation occluded

When the forearm was placed in the plethysmograph the position and shape of the gross pressure-volume curve depended upon the presence or absence of circulation as shown in Figure 2, curves B and C respectively. In this observation the forearm of a normal male subject was placed in the plethysmograph as described under "General Methods," using the 250 cc. burette. The temperature of the forearm and plethysmograph was 34° C., while room temperature was 29° C. One narrow pneumatic cuff was wrapped around the wrist just distal to the plethysmograph and another as high as possible on the upper arm just distal to the axilla.

At zero time the wrist cuff was inflated to 300 mm. Hg to exclude the flow of blood to and from the hand. At the end of one minute a burette reading was recorded and from it, as a zero point, succeeding changes in volume were computed. Pressure was first raised to 5 mm. Hg and a relatively large volume of water moved rapidly from the burette into the plethysmograph. Since equilibrium with the forearm was reached more slowly than with the glass cylinder, one minute was allowed to elapse before reading the gross change in volume corresponding to an external pressure of 5 mm. This volume having been recorded as 65 cc., pressure was raised to 10 mm. Hg, at which pressure the volume 74 cc. was reached after one minute, and so on by steps until a pressure of 100 mm. Hg was attained as shown in the completed curve B of Figure 2. The pressure within the plethysmograph was then reduced to zero and the wrist cuff was deflated.

Method I. At 0:00 time the wrist cuff was inflated to 300 mm. Hg; the pressure in the plethysmograph-burette system was elevated to the desired pressure as before, but in addition, the brachial vessels were simultaneously occluded by inflating the cuff on the upper arm to 300 mm. Hg. The volume of the forearm diminished rapidly and again the rate of change became very slow at the end of 2 minutes. At time 2:00 a burette reading was recorded and the arm cuff was deflated at time 2:00 to release circulation. As in Method II, two final readings were then made, *viz.*, the peak volume reached during reactive hyperemia (time 2:10 to 2:15) and the final volume at the end of one minute (time 3:00). The wrist cuff was then deflated and pressure in the plethysmograph was reduced to atmospheric level. A rest period of 3 minutes preceded the next determination.

Subtracting the reading taken when the vessels were collapsed at time 2:00 from those taken at the height of hyperemia indicated, for each external pressure, the increase in the volume of the forearm produced when the previously collapsed blood vessels were again distended by the *vis a tergo* of arterial pressure acting against the pressure imposed upon the surface of the forearm. The values agreed quite closely with those obtained by Method II under normal conditions but deviations were noted under certain abnormal conditions.

Computation of forearm volume. The volume of the segment of forearm enclosed by the plethysmograph was computed according to the methods described previously (6). Dividing the observed changes in forearm volume by the total volume of the segment, reduced the recorded changes in volume to cc. per 100 cc. of forearm tissue and permitted comparing vascular volumes in forearms of different sizes.

Figure 3 illustrates the effect of external pressure on "dynamic vascular volume" of the forearm. The upper half shows, for 4 subjects, the increase in volume produced by filling of the blood vessels after prior collapse (Method III), while the lower half shows the diminution in forearm volume produced by emptying of the blood vessels when circulation was stopped (Method I). As might be expected, the vascular volumes measured during reactive hyperemia, by Methods II and III, were quite similar and always slightly greater than those measured during resting conditions, by Method I. In both groups vascular volume was greatest when external pressure was between 15 and 35 mm. Hg. Though similar in this respect, some curves exhibited a very definite peak, while the highest readings of others took the form of a double peak or even

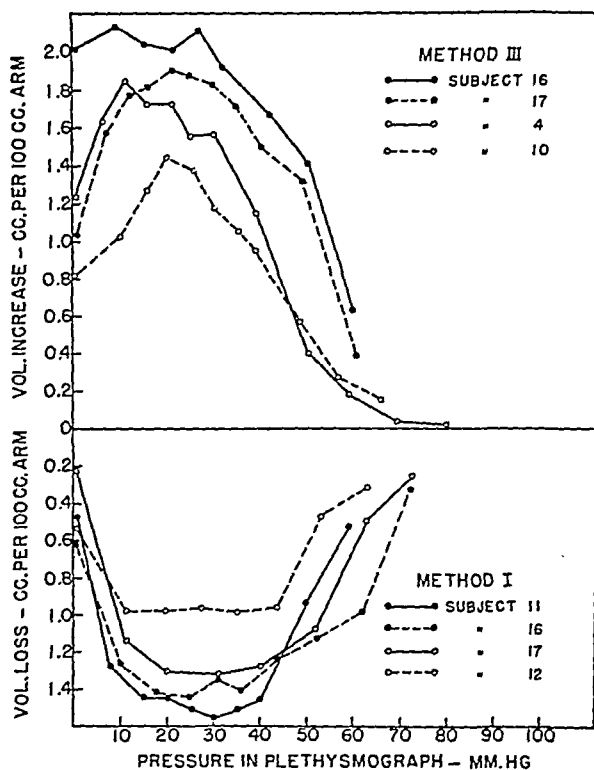


FIG. 3. SHOWING PRESSURE-VOLUME CURVES FOR 4 SUBJECTS

By Method I (emptying of vessels when circulation is stopped) and Method III (filling of vessels when circulation is resumed).

a plateau in which it was impossible by simple inspection to determine any one external pressure at which the change in volume was greatest.

When the same subjects were tested repeatedly the general relationship between external pressure and volume change remained very constant when the curve was viewed as a whole. However, minor differences in configuration indicated that, owing to experimental errors, determination of a single pressure which uniformly produced maximal change in vascular volume could not be accomplished accurately by simple inspection.

It was found that subjective interpretation could be avoided entirely and that consistent values appeared in the same or different individuals if the upper quarter of each curve was made the basis for computation. Therefore, as shown in Figure 4, a transverse line was drawn across the curve at a level one-quarter of the distance from the greatest volume observed to the base-line.

The two points at which this line crossed the pressure-volume curve indicated the limits of external pressure between which the upper quarter or peak segment of the curve was situated. When a perpendicular was erected from the midpoint of this horizontal line, it divided the area of the summit approximately in half. The external pressure opposite which this perpendicular falls will be referred to in the remainder of this paper as P_{mvc} , or "that external pressure at which the *vis a tergo* of the circulation is able to keep open the greatest or maximal collective vascular volume." Comparisons of this P_{mvc} with actual peaks or with plateau zones of individual or average curves indicated that this method, in addition to being entirely objective, had less error than inspection, measurement of slopes, and other types of analysis.

Figure 4 shows, in addition, the composite pressure-volume curves and composite P_{mvc} determined from the data for 20 normal subjects obtained by Methods I, II and III when the temperature

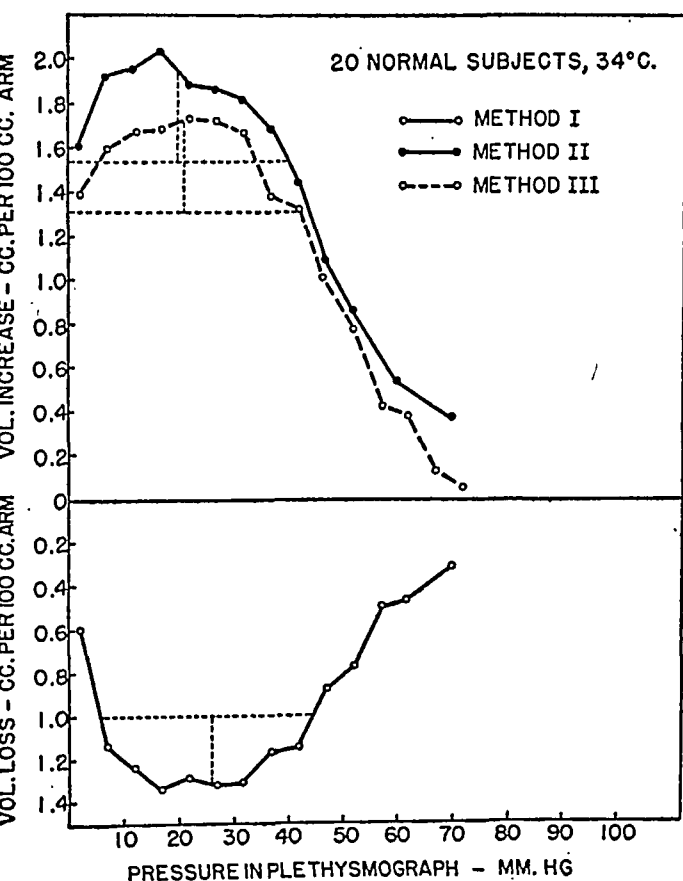


FIG. 4. SHOWING AVERAGE PRESSURE-VOLUME CURVES FOR METHODS I, II AND III, IN 20 SUBJECTS
Also illustrates manner of computing P_{mvc} .

in the plethysmograph was 34° C. Table I gives the ages, blood pressures, individual values of P_{mvc} and maximal volume changes observed in these same subjects. The values for Method III were obtained on different days from those for Methods I and II, and in some cases several weeks later. The average values of P_{mvc} were 26.9, 20.7, and 21.3 mm. Hg by Methods I, II, and III, respectively, while the corresponding average maximal volume changes were 1.38, 2.03 and 1.82 cc. per 100 cc. of forearm. The range of individual values for P_{mvc} was greater by Methods I and II than by Method III, the respective standard deviations being 3.79, 3.38 and 2.48. The differences between the mean values for P_{mvc} of Methods I and III and between those of Methods I and II are statistically significant, but the difference between the means of Methods II and III, both related to vascular filling, are insignificant. Corrections for smallness of the samples were made in these computations. The explanation of the minor differences observed will be discussed below. Despite slight differences, however, the pressure-volume curves obtained by 3 different techniques indicated that external pressures exceeding 20 to 35 mm. Hg reduced "dynamic vascular volume" significantly and that the external pressure corresponding to the summit of the pressure-volume curves, or P_{mvc} , had roughly the same order of magnitude as capillary blood pressure measured directly by micro-injection. To test the effect of changing the pressure in the minute vessels, capillary pressure was elevated by known grades of venous congestion, and "dynamic vascular volume" and P_{mvc} were determined under these conditions.

4. Effects of venous congestion on the pressure-volume curve and P_{mvc}

Venous pressure in the forearm was elevated continuously to the desired level by inflating a large pneumatic cuff, 50 cm. long and 12 cm. wide, wrapped about the upper arm as near the axilla as possible. To maintain this position and to conserve space, this wide cuff and the narrow cuff for occluding circulation were enclosed in a common cloth casing with two compartments so that the larger cuff both surrounded, and extended distally from, the lower edge of the smaller cuff. Constant pressure in the wide congesting cuff was insured by connecting it with a slow stream of compressed air, passing through a T-tube and standpipe, the latter immersed to the required depth in a column of water. As described

TABLE I

*P_{mve}** and maximum change in vascular volume determined by Methods I, II and III for 20 normal subjects at 34° C.

Subject number	Sex	Age	Arterial blood pressure	Method I		Method II		Method III	
				<i>P_{mve}</i>	Maximum volume change	<i>P_{mve}</i>	Maximum volume change	<i>P_{mve}</i>	Maximum volume change
				mm. Hg	cc. per 100 cc. forearm	mm. Hg	cc. per 100 cc. forearm	mm. Hg	cc. per 100 cc. forearm
1	M	39	122/82	20.0	2.07	19.5	2.67	19.0	2.23
2	M	20	110/66	21.5	1.74	16.0	2.59	18.0	1.78
3	M	20	104/62	22.5	1.72	21.0	2.48	17.0	2.28
4	M	23	108/64	22.5	1.68	16.0	2.42	18.0	1.85
5	F	27	92/64	24.0	1.20	21.0	1.65	19.0	1.60
6	M	21	110/70	24.0	1.71	18.5	1.87	23.5	1.62
7	M	29	112/70	25.0	1.46	18.5	1.92	20.0	1.87
8	M	27	120/75	25.0	1.39	18.5	2.04	25.0	1.86
9	M	28	110/70	25.5	1.14	18.0	1.65	19.5	1.58
10	F	28	110/74	26.5	0.95	20.5	1.65	23.0	1.44
11	M	26	116/74	26.5	1.55	21.0	2.15	22.0	1.80
12	F	30	100/60	26.5	0.99	19.0	1.89	20.0	1.72
13	F	21	98/62	27.0	1.45	18.0	2.02	20.0	1.87
14	M	31	126/74	30.0	1.26	23.0	1.81	25.5	1.78
15	M	31	114/68	30.0	1.15	22.0	1.95	22.5	1.68
16	M	23	130/80	31.0	1.45	24.0	2.36	22.0	2.14
17	M	24	122/70	31.5	1.32	28.0	1.78	23.5	1.90
18	F	27	108/70	32.5	0.86	21.0	1.84	25.5	1.79
19	M	30	118/80	33.0	1.36	28.0	2.06	22.0	1.61
20	M	23	120/74	33.0	1.26	23.0	1.81	21.0	1.98
Averages				26.9	1.38	20.7	2.03	21.3	1.82
Range of <i>P_{mve}</i> , mm. Hg				20.0-33.0		16.0-28.0		17.0-25.5	

* *P_{mve}* = that external pressure at which the *vis a tergo* of the circulation was able to keep open the greatest collective vascular volume.

previously (5), elevating venous pressure in the vertical forearm to a given level, *e.g.*, 30 mm. Hg, required that the cuff on the horizontal upper arm be inflated to the desired level, *e.g.*, 30 mm. Hg, plus an additional increment equal to the hydrostatic pressure of the column of blood in the vertical veins of the forearm. This increment, determined separately for each observation, was always between 16 and 17 mm. Hg. In the discussion, tables and figures below, only the effective venous pressure in the forearm segment is mentioned, the corresponding armlet pressure being between 16 and 17 mm. Hg greater.

Figure 5 shows the effect of continuous elevation of venous pressure on the pressure-volume curve and *P_{mve}* obtained by Method II in one subject. *P_{mve}* was determined in the usual manner and is shown by the figure near each curve. As venous pressure, and therefore capillary blood pressure, were increased, the curves became lower, moved toward the right, and *P_{mve}* became higher.

When the effective venous pressure in the forearm was zero, *P_{mve}* by Methods I and II (Table II) was within the range observed in the uncon-

gested arms of normal subjects. It is noteworthy that at this venous pressure the wide pneumatic cuff was inflated to a pressure of 16 or 17 mm. Hg and that the veins in the upper arm were distended to some extent even before circulation was shut off completely. The left limb of the curve was more complete than usual but *P_{mve}* was still in the normal range, indicating that venous filling *per se* did not distort *P_{mve}* greatly.

As effective venous pressure became higher, *P_{mve}* exceeded the imposed venous pressure markedly with the lower grades of venous congestion, and less conspicuously with higher grades of congestion. At the highest level, 50 mm. Hg, *P_{mve}* in only one observation on one subject (female) was lower than the imposed venous pressure by 4 mm. Hg; but, in this one instance, the pressure in the congesting cuff (67 mm. Hg) exceeded diastolic pressure by 5 mm. Hg so that interference with arterial inflow probably prevented complete congestion in the time available between separate ob-

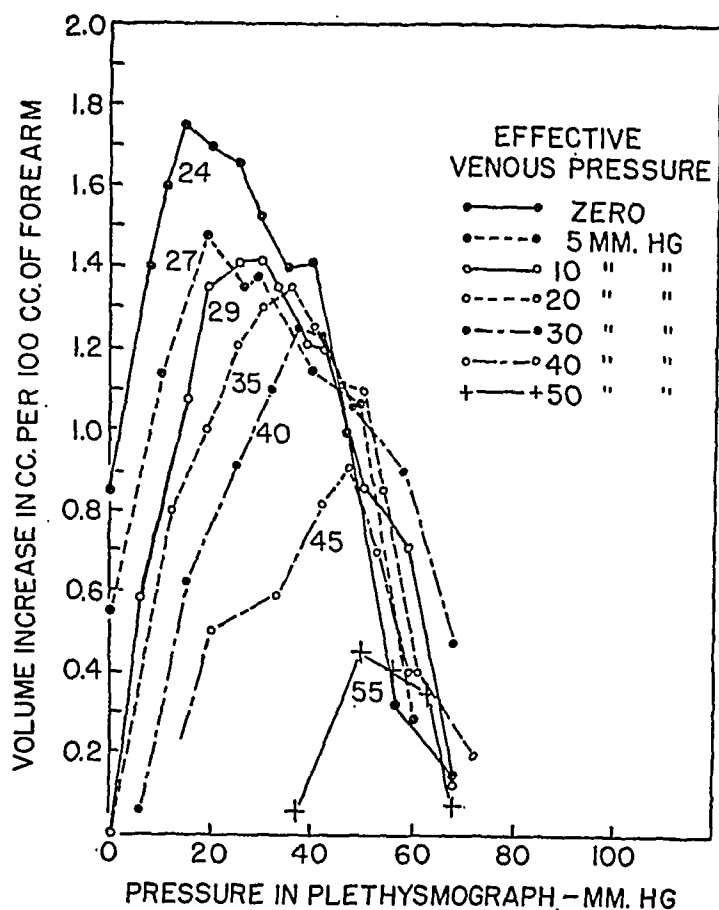


FIG. 5. SHOWING EFFECT OF GRADED VENOUS CONGESTION ON PRESSURE-VOLUME CURVES AND P_{mvc} OF ONE SUBJECT BY METHOD II

servations. Similar inconsistencies were observed when brief intermittent venous congestion was used.

Comparison of the results by Methods I and II (Table II) indicates again that vascular filling by reactive hyperemia produces slightly greater volume changes and slightly lower P_{mvc} than does simple emptying of vessels when circulation is normal. As would be expected, these slight differences disappear as the level of venous pressure increases and as the capillary bed becomes more completely distended.

With Method III, however, the pressure-volume curves were bizarre and unreadable when effective venous pressure was raised by continuous congestion to 10 mm. Hg or more. While Methods II and III were similar in measuring volume change during reactive hyperemia, they differed in the degree to which the arm was emptied of blood before volume changes were measured. In Method II, pressure in the plethysmograph was elevated 2 minutes before the brachial artery and veins were

occluded. The forearm was therefore partially or completely emptied of blood before the veins of the upper arm were occluded. In Method III, the brachial artery and veins were occluded by inflating the axillary cuff at the same time as pressure in the plethysmograph was raised. The latter pressure then displaced blood into the already distended veins of the upper arm, creating there a pressure considerably above that in the congesting cuff. Deflation of the narrow occluding cuff at the axilla allowed varying quantities of blood to pass immediately under the wide congesting cuff and the volume of the forearm diminished suddenly by an irregular and unpredictable amount, owing to emptying of the overdistended veins. Under these conditions the increase in volume by reactive hyperemia was diminished or even nullified by the simultaneous decrease in volume due to sudden venous emptying. The pressure-volume curves were entirely unreliable as recorded in Table II.

The results indicate that while the limited venous reservoir in the upper arm produces little distortion, even by Method III, when venous pressure is normal, this shorter method introduces serious errors and inconsistencies when venous pressure is high. It would seem therefore that in this situation Methods I and II are more reliable. The specific values for P_{mvc} will be compared with directly determined capillary and venous blood pressures below.

5. The effects of position of the forearm on the pressure-volume curve and on P_{mvc}

The effects of position of the hand on venous (9) and capillary blood pressure (1) are known from previous direct measurements. Changing the position of the forearm relative to the heart changed the pressure-volume curves as illustrated in Figure 6. The number and arrow with each curve gives the corresponding value for P_{mvc} .

To raise the mid-point of the forearm 30 cm. above the suprasternal notch (plus 30 cm., Figure 6), the subject was seated in a low chair, the arm was supported vertically, and the plethysmograph was raised as high as possible on its standard. At 0 cm. (Figure 6), the middle of the forearm then being opposite the suprasternal notch, the position was essentially that used routinely and described previously under Methods. To depress the forearm 12 cm. below the suprasternal notch (minus 12 cm.,

Figure 6) the subject was seated and the arm placed horizontally at the side. Maximal dependency of the forearm (minus 35 cm., Figure 6) was obtained by hanging the arm downward and vertically at the side of the seated subject. The plethysmograph was inverted with the wider aperture uppermost. In each position the burette, the burette reservoir, water outlet and manometer (Figure 1, I, K, E, M) were, as usual, placed level with the upper diaphragm of the plethysmograph. When the heavy iron standard could not be used, the plethysmograph, hand and elbow were supported by sandbags. The relation of the forearm to the plethysmograph in these experiments was less stable than in the routine arrangement described in previous sections, but remained constant enough to permit satisfactory readings.

In three determinations by Method III, when the mid-point of the vertical plethysmograph was 30 cm. above the suprasternal notch, the greatest changes in volume ranged from 1.32 to 1.60 cc. per 100 cc. of arm and were therefore essentially the same as those observed at heart levels, while P_{mrc} was lowered to 10, 11 and 12 mm. Hg. At 0 cm. with the middle of the forearm opposite the suprasternal notch, P_{mrc} was 22 mm. Hg but when

the forearm was depressed to between 12 and 13 cm. below its usual position, P_{mrc} rose to 34 and 34.5 mm. Hg by Method II. The pressure-volume curve in this latter position resembled in configuration and amplitude those resulting from an effective venous congestion of 10 mm. Hg in the routine position.

When the forearm was situated 20 cm. below the suprasternal notch, P_{mrc} was 39 and 41.5 mm. Hg. Increasing this distance to between 35 and 40 cm. elevated P_{mrc} to 52 and 50 mm. Hg by Method I and 44 mm. Hg by Method II. The greatest dependency obtainable was 45 cm. and P_{mrc} by Method I then became 72, 68 and 68 mm. Hg and 51, 52 and 57 mm. Hg by Method II. At these levels the maximal volume changes were greater than those noted when venous pressure was elevated to the same degree by means of the pneumatic cuff, and the curves were less regular because the relation between the forearm and the plethysmograph could not be kept absolutely constant. As with high grades of venous congestion

TABLE II
Effects of venous congestion on P_{mrc} and maximal change in vascular volume at 34° C.

Effective venous pressure	Method I			Method II			Method III			Average Methods I, II	
	Subject number	P_{mrc} mm. Hg	Maximal volume change cc. per 100 cc. forearm	Subject number	P_{mrc} mm. Hg	Maximal volume change cc. per 100 cc. forearm	Subject number	P_{mrc} mm. Hg	Maximal volume change cc. per 100 cc. forearm	P_{mrc} mm. Hg	Maximal volume change cc. per 100 cc. forearm
Zero*	5 15	29.0 29.0	1.05 1.05	5 15	21.0 24.5	1.41 1.75	5 7	28.0 32.0	1.44 1.20	25.5	1.32
5 mm. Hg	5 15	29.0 34.0	1.09 0.86	5 15	22.0 27.0	1.52 1.48	5 7	Unreliable 30.0	1.47	28.4	1.28
10 mm. Hg	5 15	33.0 37.5	0.93 0.83	5 15	31.0 29.0	1.31 1.42	5 5 15 21	Unreadable Unreliable Unreliable Unreliable		32.6	1.12
20 mm. Hg	5 15	33.0 38.5	1.10 0.81	5 15	32.0 35.0	1.35 1.36	5 21	Unreadable Unreadable		34.6	1.16
30 mm. Hg	5 15	39.5 48.0	0.99 0.78	5 15	35.0 40.5	0.99 1.26	5 7 15	Unreadable Unreadable Unreadable		40.8	1.00
40 mm. Hg	5 15	44.0 49.0	0.51 0.68	5 15	44.0 45.0	0.51 0.91	5 15	Unreadable Unreadable		45.5	0.65
50 mm. Hg	5 7 15	46.0 55.0 57.0	0.43 0.49 0.42	5 7 15	Unreliable 55.0 55.0	0.51 0.46	5 15	Unreadable Unreadable		53.6	0.46

* Hydrostatic pressure in vertical forearm veins balanced by equal pressure in congesting cuff.

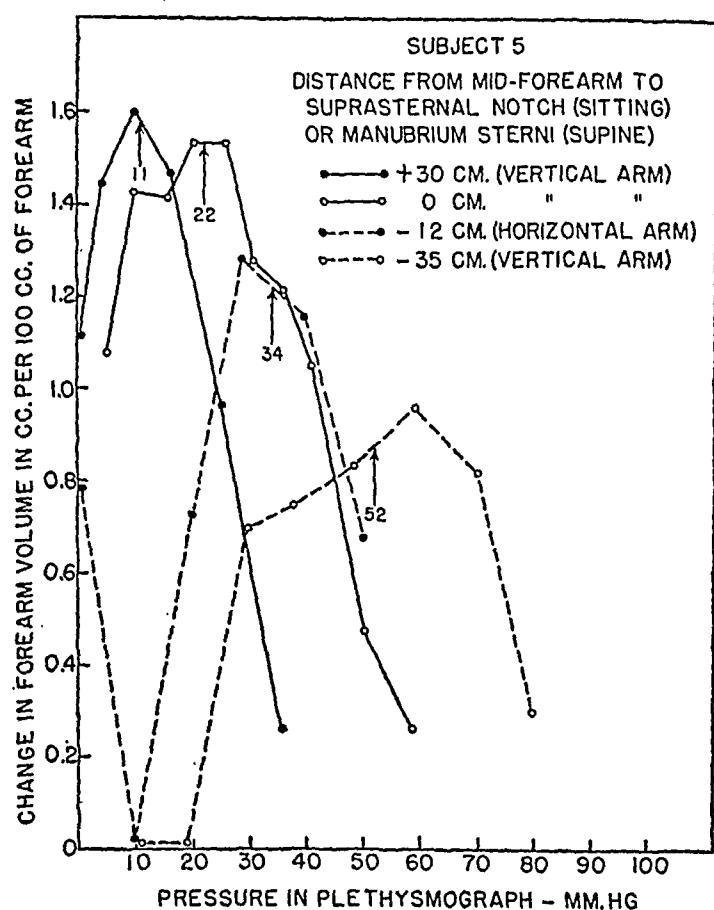


FIG. 6. SHOWING EFFECT OF POSITION OF THE FOREARM ON PRESSURE-VOLUME CURVES AND P_{mvc}

by the pneumatic cuff, Method III yielded bizarre curves in the lowest positions, without a clear maximum, due, it is believed, to the same over-distention of the arm veins which was described in the previous section.

It appears that P_{mvc} is diminished by elevating the forearm and increased by depressing the forearm and that in these positions the observed values of P_{mvc} are of the same order of magnitude as those observed with corresponding venous congestion.

6. Comparison of total vascular volume and "dynamic vascular volume" in relation to venous congestion

Venous congestion, as is well known, increases total vascular volume because the veins and capillaries are distended by greater internal pressure. Since increasing venous congestion progressively lowered, rather than raised, the peaks of the pressure-volume curves described in the previous two sections, it is obvious that something less than total vascular volume is being measured.

To this volume we have applied the term "dynamic vascular volume" for three reasons: (a) it exists when arterial flow is present and disappears when arterial and venous blood flow are interrupted; (b) it is affected in characteristic manner by external pressure as shown by the preceding pressure-volume curves; and (c) venous congestion, while increasing total vascular volume, induces stasis, decreases blood flow and also decreases this "dynamic vascular volume." The combined effect of venous congestion and external pressure on total vascular volume is shown in Figure 7, to contrast with the curves of "dynamic vascular volume" shown in Figures 3 to 6.

In these observations the forearm was in the usual position with narrow pneumatic cuffs around the wrist and as close as possible to the axilla. Just below the axillary cuff was wrapped a separate wide pneumatic cuff for producing venous congestion. The double cuff could not be used because inflation of the small cuff prevented entrance of air into the large cuff. At zero time, the wrist cuff was inflated to 300 mm. Hg as usual; simultaneously the pressure in the plethysmograph was elevated to 200 mm. Hg for the purpose of collapsing and evacuating the blood vessels in the forearm as completely as possible. Because the veins in the arm were still open, this blood entered the systemic circulation. At 2:00 minutes the cuff at the axilla was inflated to 300 mm. Hg to keep the forearm bloodless. Immediately thereafter the plethysmograph pressure was reduced to 0 or 10 or 20, etc., mm. Hg, and the large congesting cuff was simultaneously inflated to the desired venous pressure, 0 or 10 or 20, etc., mm. Hg. At 3:00 minutes the volume of water in the burette was recorded; circulation was then released. The peak volume reached during reactive hyperemia was recorded. The difference between the 2 readings indicated total vascular volume during various combinations of venous congestion and external pressure as shown in Figure 7, the vessels having been emptied as much as possible previously.

At all venous pressures the greatest volume was observed when external pressure was zero, and all these volumes were greater than those observed in any of the previous experiments. When effective venous congestion was zero, the pressure-volume curve passed through a slight plateau at pressures of 10 to 30 mm. Hg, and then fell off steeply at higher external pressures. Increasing congestion to 10 mm. Hg merely emphasized the plateau, and congestion of 20 mm. Hg shifted the plateau toward higher pressures. Congestion to 30 mm. Hg produced definite distortion to a more

nearly sigmoid curve which became completely sigmoid when the congestion reached 40 mm. Hg. It is of interest to recall that Lewis (7) found 30 mm. Hg the lowest congesting pressure which could produce detectable reactive hyperemia in the forearm, suggesting that this pressure was the lowest to interfere with capillary blood flow.

This indicates that, even when "total vascular volume" is measured, the configuration of the pressure-volume curve is altered distinctively when external pressure reaches that level believed to exist in the minute vessels, providing venous congestion is not great enough to obliterate the normal gradient of blood pressure in the capillary network. When congestion is extreme and extends throughout the capillary bed, the curve is that to be expected when external pressure acts upon an elastic system which is distended by uniform pressure in all its parts.

That this total vascular volume differs slightly from "dynamic vascular volume," even when no venous congestion is present, is illustrated by the insert in Figure 7 in which these two pressure-volume curves are superimposed. They are similar above 20 mm. Hg but below that level "dynamic vascular volume" becomes less, probably because the arm has not been previously emptied of blood. A comparison of Figures 5 and 7 indicates, however, that "dynamic vascular volume" affords a more revealing estimate of the interaction of *vis a tergo*, effective intravascular pressure, and volume of blood actually in movement, while excluding from measurement that volume of blood which is in stasis due to congestion.

7. Comparison of P_{mve} with direct determinations of capillary blood pressure and venous blood pressure

The method of determining P_{mve} was adopted for theoretical reasons before, and not after, the detailed comparison in Table III was made. Though the pressure-volume curves and P_{mve} are entirely objective values, nevertheless the meaning of this or any other arbitrarily chosen criterion must be tested by comparing its results with those of the micro-injection method, the latter being the most accurate procedure so far available for determining capillary blood pressure (3). As shown in Table III, in the resting state with the forearm

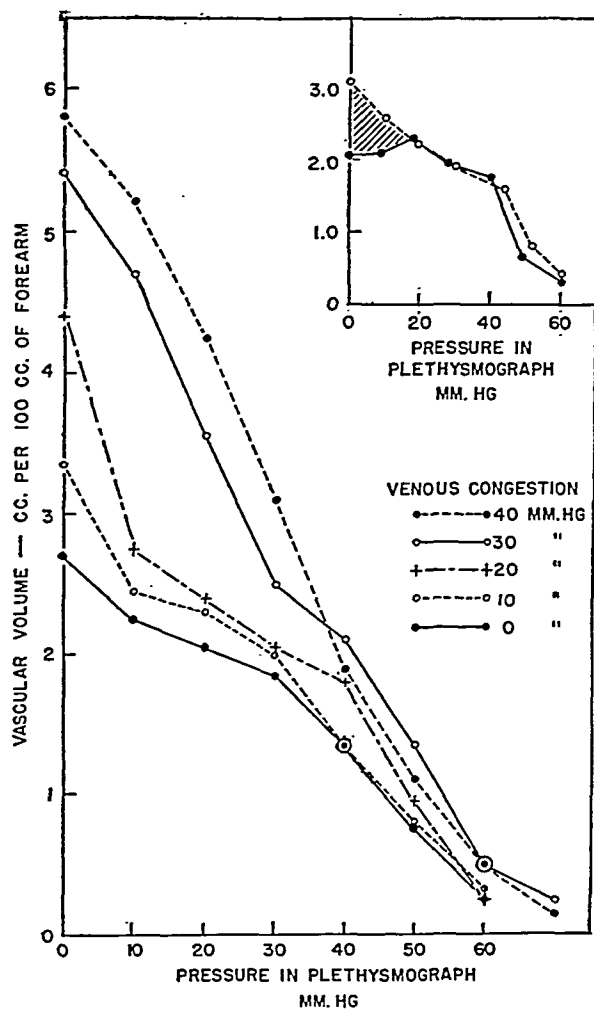


FIG. 7. SHOWING EFFECT OF VENOUS CONGESTION AND EXTERNAL PRESSURE ON TOTAL VASCULAR VOLUME AVERAGE FOR 2 SUBJECTS

Inset compares total vascular volume (circles and broken line) and "dynamic vascular volume" (dots and solid line) at normal venous pressure.

at heart level, average P_{mve} ranged, according to method used, from 17 to 33 mm. Hg with average values of 21 to 27 mm. Hg. By Method II, P_{mve} ranged from 16 to 28 mm. Hg with 21 mm. Hg as average, while the figures by micro-injection (1) ranged from 6 to 48 mm. Hg, with 22 mm. Hg as average. Since the plethysmographic measurements included all the peripheral vessels, the micro-injection values for both limbs of the capillary loop have been grouped together. Whether mean values or extremes are compared, P_{mve} in the normal subject has the same order of magni-

TABLE III
Comparison of P_{mve} with capillary blood pressure determined directly (1, 2, 3)

	Method	P_{mve}	Capillary blood pressure by micro-injection	Reference
Resting state with extremity at heart level	I II III	mm. Hg 27(20-33) 21(16-28) 21(17-26)	mm. Hg 22(6-48)	(1)

	Venous pressure	P_{mve}	Venous pressure	Capillary blood pressure	
	mm. Hg	mm. Hg	mm. Hg	mm. Hg	
Elevated venous pressure (congestion)	10	29-37	10	25, 35	(2)
			12	25	(2)
	20	32-39	24	41	(2)
	30	35-48	27	35	(2)
	40	44-49	40	48-50	(1)
			45	47	(3)
	50	55-57	47	50-56	(3)
			52	58-68	(3)
			60	60-76	(1, 3)

	Position of forearm	P_{mve}	Position of finger	Capillary blood pressure	
	cm.	mm. Hg		mm. Hg	
Change in position relative to suprasternal notch (plus above, minus below)	+30	10-12	-32	6-26	(1)
	0	21-27	0	7-44	(1)
	-12	27-35			
	-20	39-42	18	17-52	(1)
	-35	44-52			
	-40	44-50	40	30-62	(1)
	-45	51-68			

tude as the directly determined values of capillary blood pressure.

The same holds true when the comparison is extended to the observations in which capillary blood pressure was diminished by raising the forearm, or increased by venous congestion and lowering the forearm. However, even when the observations of Landis (1), Fahr and Ershler (2), and Eichna and Bordley (3) are pooled, they are not numerous enough to justify computing averages, so that only the range of individual determinations is given in the lower 2 sections of Table III. Elevation of venous pressure to 10 or 20 mm. Hg affected capillary blood pressure and P_{mve} less than did greater increments of venous

pressure. In both series the amount by which the determined values exceeded venous pressure was greater with mild congestion than with severe congestion, as would be expected because of the gradient of pressure believed to exist in the capillary network.

In the present study, venous pressure was also measured directly in the horizontal arm at the antecubital space in 5 subjects before and after circulation was occluded by the axillary cuff. Before occlusion, venous pressure ranged from 7.5 to 9.0 mm. Hg, averaging 8.0 mm. Hg. In agreement with Kendrew (8) it was found that inflation of the narrow cuff below the axilla increased venous pressure, which then ranged from 10.5 to 16.5 mm. Hg, with an average of 13.5 mm. Hg. In a single observation on one subject the venous pressure taken twice in succession in the occluded arm was 20.0 and 24.5 mm. Hg while in a third measurement a few moments later it was 15.5. Since these first two exceptionally high values were followed by one in the more usual range it seems likely that the movement of fluid from the manometer to the vein was not entirely free and they have been excluded from the above average. Release of the cuff, after 2 minutes of complete ischemia, reduced venous pressure at the antecubital space immediately to an average of 8.5 mm. Hg, even during reactive hyperemia.

Venous pressure could not be measured in the forearm itself in the vertical position. However, this pressure in the vertical forearm would certainly be considerably less than the 13.5 mm. Hg observed in the antecubital space. These estimations of venous pressure may explain in part why P_{mve} by Method I amounted to 27 mm. Hg as compared to 21 mm. Hg by Methods II and III. In Method I the change in vascular volume of the forearm was measured while the axillary cuff was inflated, and any blood expressed from the forearm met an average initial resistance of 13.5 mm. Hg rather than the normal venous pressure of 8.0 mm. Hg. On the contrary, in Methods II and III the axillary cuff was deflated, the veins were open, and the venous pressure was 8.5 mm. Hg, or lower than before by 5.0 mm. Hg. The discrepancy between P_{mve} by Method I and Methods II and III is almost exactly equal to this difference in venous pressure.

The comparison with venous pressure can be extended also to the measurements made when the forearm was elevated above, or depressed below, heart level. In the former position Carrier and Rehberg (9) found venous pressure was 3.5 mm. Hg, whereas P_{mrc} determined plethysmographically ranged from 10 to 12 mm. Hg. Therefore, in whatever position the forearm was placed P_{mrc} was definitely higher than venous pressure and had the same order of magnitude as capillary blood pressure.

DISCUSSION

The significance of the pressure-volume curves and of P_{mrc} , as described in this paper, depends upon (1) the collective volume of the capillary network compared to that of the venules and arterioles; (2) the accuracy with which pressure is transmitted from the plethysmograph, through the tissues, to the blood within the vessels; (3) the magnitude of errors arising from the effect of external pressure on extravascular fluid; (4) the effect of low external pressures on the volume of the capillary network by reason of passive congestion produced by partial compression of veins; (5) the part played by the capacity of veins in the upper arm during the occlusion of circulation; and (6) the relation of the pressure-volume curves and P_{mrc} to known changes in venous and capillary blood pressures under various conditions.

(1) As blood flows through the peripheral vessels, it enters first the arteries and larger arterioles where cross-sectional area is small, pressure is high, and flow is rapid. In the capillaries, cross-sectional area becomes relatively enormous while pressure and rate of flow diminish. Finally in the veins, the cross-sectional area again diminishes, pressure reaches its lowest level, and the rate of flow once more increases. Concerning the comparative collective volumes of these sections of the vascular tree in the extremities, there is little quantitative information, but from what is known of circulation time and the total capillary volume, it can hardly be questioned that the minute arterioles, the true capillaries and the venous capillaries have a total volume greater than that of the other subdivisions of the vascular tree. From photomicrographs of the vessels in the frog's mesentery previously published (10), computation

shows that one terminal arteriole gave rise to a series of arteriolar capillaries whose total volume was at least ten times as great as that of the parent vessel, and it seems likely that venous capillaries would contribute an equal, if not greater, volume.

When pressure is applied externally to a system as complicated as this, there must follow a series of changes which cannot be divided into definite stages because they merge gradually into each other. If increasing pressure is applied to the surface of the skin, for instance by a capsule, the first change observed is a flattening of veins while blood flow still continues through the partially collapsed vessels. It has been shown (11) that even this partial collapse produces slight but definite passive congestion in the capillary area drained by the vein, even though the applied external pressure is still below venous pressure. As external pressure is increased further, to between 5 and 15 mm. Hg, the skin blanches owing to compression of the subpapillary venous plexus. Higher pressures, possibly in the vicinity of 15 to 45 mm. Hg, compress the true capillaries and still more pressure is required to compress larger arterioles, *e.g.*, in the retina. While many studies describe the visible effects of external pressure on these vessels, no observations have been found which measure effects on collective vascular volume.

Because the peripheral blood vessels present various combinations of pressure and volume, it was expected that the pressure-volume curves might show a characteristic configuration. In simplest terms low external pressures, just sufficient to compress the veins partially, might produce either a relatively small decrease in vascular volume or, if capillary congestion and passive distention were significant, a slight increase in net vascular volume. In either case, however, when external pressure becomes great enough to impinge on the capillary vessels and the smallest arterioles, vascular volume should decrease conspicuously. Pressures above this level would make the forearm relatively bloodless because only the larger arterioles and arteries could remain filled. Conversely, if external pressure were gradually lowered from 120 mm. Hg, the most striking increase in vascular volume would be expected when external pressure became low enough to

been due to the previously mentioned limited capacity of the veins in the upper arm, or to distention of the relaxed venules and veins by the sudden inrush of blood in reactive hyperemia (7). In any case, it does not explain entirely the 6 mm. difference in P_{mvo} because the right limbs of the curves are also displaced toward lower pressures by mild reactive hyperemia. The difference is real since it was observed uniformly in repeated trials on the same subjects at widely separated time intervals. The configuration of the pressure-volume curves was also the same when pressures were applied in descending or ascending order, or even when no regular sequence was followed.

In the observations described above capillary blood pressure was elevated by venous congestion and the rise in P_{mvo} was associated with a reduction of "dynamic vascular volume." This might lead to the supposition that these two quantities were related inversely under all conditions. Preliminary studies have indicated that cooling of the forearm reduces both P_{mvo} and "dynamic vascular volume." Conversely, exercising the forearm muscles raises P_{mvo} beyond the highest normal limits in most instances, while vascular volume is usually greater than normal. It appears therefore that under different conditions P_{mvo} and "dynamic vascular volume" can vary independently.

In using this procedure to study the peripheral circulation under abnormal conditions it would seem advisable to use both Methods I and II together rather than either one separately. Preliminary observations in patients with hypertension and with reduced blood volume indicate that under abnormal conditions the pressure-volume curves obtained by these two methods differ more than they do in normal subjects. In these two conditions heightened arteriolar tone changes the position of the right limb of the pressure-volume curves, but this distortion is lessened even by mild reactive hyperemia.

Finally, this plethysmographic method of measuring the effect of external pressure on the vascular volume and the collective intravascular pressure in the minute vessels of the forearm has the weakness inherent in any "indirect method" of measurement because the criterion is an arbitrary

one. The pressure-volume curves seem to be more reproducible than the purely visual criteria used in other indirect methods (3) and they have been shown to be modified characteristically when tested during venous congestion of known grade. The method is objective, includes large numbers of minute vessels, and avoids errors due to the proximity of the arteriovenous anastomoses. With the addition of graphic recording of volume changes, it is being used for studying the minute vessels in clinical conditions in a manner quite impossible with the direct micro-injection method.

SUMMARY

The pressure plethysmograph was used to determine the effect of graded external pressure on the vascular volume of the forearm, for the purpose of determining the usefulness of this procedure in estimating the blood pressure in the minute vessels collectively.

With external pressures ranging from 0 to 90 mm. Hg, pressure-volume curves were determined in 20 normal subjects (a) by suddenly arresting the circulation to the forearm and measuring decrease in volume, and (b) by releasing circulation suddenly after prior arrest and measuring increase in volume during the ensuing mild hyperemia. The term "dynamic vascular volume" was used to indicate that the volume of blood in actual movement was being measured under these conditions.

In the normal forearm "dynamic vascular volumes" were greatest when external pressure was between 15 and 35 mm. Hg, becoming less at external pressures above and below this range.

To record the relation between "dynamic vascular volume" and external pressure in the form of a single numerical value, an objective method of analyzing the pressure-volume curves was adopted. The single value thus obtained was termed P_{mvo} and was defined as "that external pressure at which the *vis a tergo* of the circulation is able to keep open the greatest collective dynamic vascular volume."

P_{mvo} determined in the forearms of 20 normal subjects with the forearm segment at heart level and at 34° C. was 27, 21 and 21 mm. Hg by Methods I, II and III respectively. Reasons are given for regarding Methods I and II as the most

useful. In the normal subject the results by all three methods had roughly the same order of magnitude as average capillary blood pressure when determined directly.

This similarity between P_{mcc} and directly determined capillary blood pressure held also when the latter was reduced by elevating the forearm or increased by known venous congestion and by depressing the forearm below heart level.

With due precaution against assuming too quickly the quantitative validity of any indirect method of measuring intravascular pressure, it is suggested that the plethysmographic method may be useful in studying the volume of blood and the pressure in the minute vessels of the forearm in clinical conditions.

PROTOCOL I

Normal subject, J. E.; blood pressure 110/72 mm. Hg, December 12, 1940.

The plethysmograph-burette system was arranged as described. The plethysmograph was fitted to left forearm, small pneumatic cuffs were applied around wrist and upper arm, and water levels were adjusted to give atmospheric pressure. Temperature of the water in the bath was 34° C., and of the room, 28° C.

The pressure in the plethysmograph was elevated to 100 mm. Hg three times to settle the rubber sleeve against the forearm.

Time
Minutes, Seconds
0:00 (a) The wrist cuff was inflated to 300 mm. Hg pressure.

(b) The pressure applied to the water in the burette and the burette reservoir and thence to the forearm in the plethysmograph was 0.0 mm. Hg.

0:10 The burette reservoir (Figure 1, J) was excluded by closing stopcock Y.

2:00 The volume read in the burette was 20.2 cc. (column 3, below).

2:01 Circulation was arrested by inflating the arm cuff to 300 mm. Hg.

4:00 The volume read in burette was 26.8 cc. (column 4).

4:01 Circulation was restored by deflating the arm cuff.

4:10-4:15 The maximum (peak) volume during reactive hyperemia, read in the burette, was 13.1 cc. (column 5).

5:00 (a) The final volume read in burette was 17.5 cc. (column 6).

(b) The wrist cuff was deflated.

(c) Stopcock Y to the burette reservoir was opened.

5:00-9:00 Rest period.
(9:00 = time 0:00 for next determination.)

0:00 (a) The wrist cuff was inflated to 300 mm. Hg pressure.

(b) Pressure applied to the water in the burette and thence to the forearm in the plethysmograph was 10 mm. Hg.

0:10 The burette reservoir was excluded by closing stopcock Y.

2:00 The volume read in the burette was 22.2 cc. (column 3).

2:01 Circulation was arrested by inflating the arm cuff to 300 mm. Hg.

TABLE IV

Protocol I completed and summarized

Time determination started	Pressure applied to surface of forearm in plethysmograph	Burette reading after 2 minutes of external pressure with circulation intact	Burette reading after 2 minutes of ischemia and continued external pressure	Burette reading at peak of reactive hyperemia, during continued external pressure	Final burette reading at end of 5th minute of external pressure	Decrease in volume during ischemia and external pressure (column 4 minus column 3)	Increase in volume during reactive hyperemia and external pressure (column 4 minus column 5)
1	2	3	4	5	6	7	8
minutes seconds	mm. Hg	cc.	cc.	cc.	cc.	Method I cc.	Method II cc.
0:00	0	20.2	26.8	13.1	17.5	6.6	13.7
9:00	10	22.2	32.6	16.9	21.5	10.4	15.7
18:00	15	22.0	32.6	17.3	21.3	10.6	15.3
27:00	20	21.2	32.0	16.4	20.5	10.8	15.6
36:00	25	21.6	32.5	17.5	21.7	10.9	15.0
45:00	30	21.8	31.8	18.6	21.0	10.0	13.2
54:00	40	21.1	29.6	16.8	19.5	8.5	12.8
63:00	50	21.0	27.1	20.5	20.5	6.1	6.6
72:00	60	23.2	26.2	23.6	23.6	3.0	2.6

Forearm measurements: circumferences, wrist 17.0 cm.

elbow 25.9 cm.

length of segment in plethysmograph, 17.3 cm.

volume (calculated), 633 cc.

- 4:00 The volume read in the burette was 32.6 cc. (column 4).
- 4:01 Circulation was restored by deflating the arm cuff.
- 4:10-4:15 The maximum (peak) volume read in burette was 16.9 cc. (column 5).
- 5:00 (a) The final volume read in burette was 21.5 cc. (column 6).
 (b) The wrist cuff was deflated.
 (c) Stopcock Y to the burette reservoir was opened.
 (d) Atmospheric pressure was restored in the burette-plethysmograph system.
- 5:00-9:00 Rest period. (18:00 = time 0:00 for next determination) (*et cetera*, successively at pressures shown in table IV.

BIBLIOGRAPHY

1. Landis, E. M., Micro-injection studies of capillary blood pressure in human skin. *Heart*, 1930, 15, 209.
2. Fahr, G., and Ershler, I., Capillary pressure in right heart failure. *Proc. Soc. Exper. Biol. and Med.*, 1938, 37, 701.
3. Eichna, L. W., and Bordley, J., III., Capillary blood pressure in man. Comparison of direct and indirect methods of measurement. *J. Clin. Invest.*, 1939, 18, 695.
4. Landis, E. M., Capillary pressure and capillary permeability. *Physiol. Rev.*, 1934, 14, 404.
5. Landis, E. M., and Gibbon, J. H., Jr., The effects of temperature and of tissue pressure on the movement of fluid through the human capillary wall. *J. Clin. Invest.*, 1933, 12, 105.
6. Krogh, A., Landis, E. M., and Turner, A. H., The movement of fluid through the human capillary wall in relation to venous pressure and to the colloid osmotic pressure of the blood. *J. Clin. Invest.*, 1932, 11, 63.
7. Lewis, T., *The Blood Vessels of the Human Skin and Their Responses*. Shaw and Sons, London, 1927.
8. Kendrew, A., The graphic registration of venous pressure in man, illustrated by some observations upon reactive hyperemia. *Heart*, 1926, 13, 101.
9. Carrier, E. B., and Rehberg, P. B., Capillary and venous pressure in man. *Scandinav. Arch. f. Physiol.*, 1923, 44, 20.
10. Landis, E. M., Poiseuilles's law and the capillary circulation. *Am. J. Physiol.*, 1933, 103, 432.
11. Landis, E. M., The capillary pressure in frog mesentery as determined by micro-injection methods. *Am. J. Physiol.*, 1926, 75, 548.

THE EFFECT OF EPINEPHRINE ON THE VOLUME OF THE BLOOD

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A previous investigation (1) has shown that as a result of a sudden rise in the venous and arterial pressures by exercise there is a prompt and definite decrease in the plasma volume. Further, moderate exertion is accompanied by a proportionate diminution in the blood volume, since the volume of the cells remains essentially the same. Only when the physical activity is severe are new cells added to the circulating volume. During the recovery period, the volume of the blood gradually returns to the resting value. It was suggested that the elevated capillary pressure produced by exercise caused increased filtration of fluid through the capillary walls, which in turn leads to a decrease in the plasma volume, and that the slight increase in cell volume during severe exercise was a result of extrusion of cells from the blood depots into the circulating blood. It was suggested that the latter was mediated through the sympathico-adrenin mechanism.

If these factors are responsible for the alterations in the volume of the blood during exhaustive exercise, the administration of epinephrine might be expected to produce a similar picture, since this drug increases the systemic pressure and contracts the blood depots. The purpose of this communication is to report the observations of the blood volume before and after the subcutaneous injection of epinephrine in normal individuals, in patients with splenomegaly, and in 2 subjects whose spleens had been removed following rupture.

METHODS AND MATERIAL

The methods employed in this investigation were the same as those described previously (1). All experiments were carried out under basal conditions. On coming to the laboratory in a post-absorptive state the subject rested in a horizontal position for 60 minutes. The azo blue dye (T-1824) was then injected intravenously and the disappearance slope for the calculation of the initial plasma volume was determined over a period of 40 minutes. By this method, changes in the plasma volume in

excess of +40 cc. and -90 cc. may be considered significant (1). Blood was also taken at intervals for cell volume, hemoglobin, and serum protein determinations. The blood and cell volumes were obtained from the plasma volume and the hematocrit value. The control venous pressure and circulation time were then measured.

Immediately after the control data were obtained, from 0.8 to 1 cc. of epinephrine (1-1000) was administered subcutaneously. Blood for serum samples and hematocrits was withdrawn from the ante-cubital vein at appropriate intervals after the injection of the drug. From the initial plasma volume and the deviation of the dye concentration of these samples from the prolongation of the disappearance slope, changes in the plasma volume were calculated. At varying intervals during the experimental period, the venous and arterial pressures and the circulation time were determined.

The above procedure was carried out on 5 young subjects with normal cardiovascular systems and one subject who was in good condition save for chronic rheumatoid arthritis. In addition studies were made on 2 individuals with polycythemia vera and on 2 normal subjects whose spleens had been removed one year previously because of an accidental injury. Prior to this study, the patients with polycythemia had been subjected to repeated therapeutic phlebotomies during a period of several years. Both subjects had palpable spleens.

RESULTS

Normal subjects. The changes noted in the plasma and cell volumes in response to the administration of epinephrine, along with other data relating to the cardiovascular system, are shown in Table I and Figure 1. Within approximately 11 minutes after the injection of the drug, there was a decrease in the plasma volume in all instances except two (G. M. and C. H.), in which the volume remained essentially the same. From this point until the termination of the experiment (45 minutes after the administration of epinephrine) the plasma volume gradually diminished, so that 17 to 24 minutes after the drug, the average decrease in plasma volume was 125 cc. or 4.1 per cent.

Fairly consistent changes were observed in the cell volume. Increases in cell volume were noted

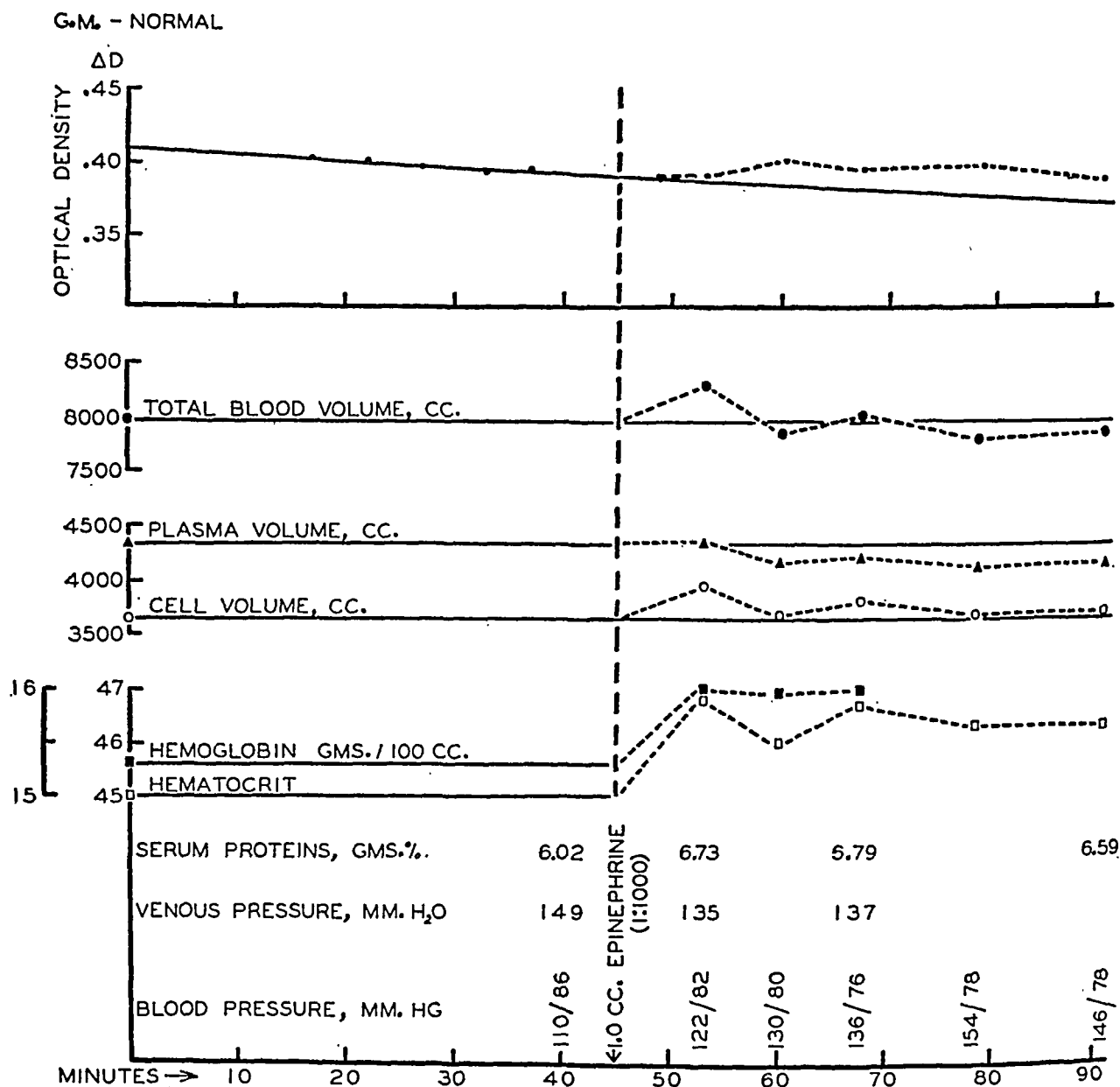


FIG. 1. THE CHANGES IN THE VOLUME OF THE BLOOD AND OTHER RELATED CIRCULATORY MEASUREMENTS BEFORE AND AFTER THE SUBCUTANEOUS INJECTION OF EPINEPHRINE IN A NORMAL SUBJECT

in 4 cases, while in 2 instances there were no significant changes (F. G. and W. P.). The increase in the cell volume was observed shortly after the injection. The gain in volume amounted to as much as 300 cc. Following the initial rise, the usual course was a gradual decrease in the cell volume so that, at the end of the experimental period, the average increase was only about one-fourth of the initial rise. Part of the increase in cell volume was due to an increase in the leukocytes.

The alterations in the blood volume were variable, since they were dependent upon the relative changes in the cell and plasma volumes. In one-half of the cases the blood volume was higher

than the control value shortly after the injection of epinephrine (6 to 11 minutes). This was due to the rather early marked increase in the cell volume. Following this the blood volume diminished somewhat as a result of the fall in the cell volume. In several cases where the decrease in plasma volume was greater than the increase in cell volume, the blood volume was less than the initial value.

These changes in the volume of the blood were accompanied by an increase in the hemoglobin per unit volume of blood, in the hematocrit, and in the viscosity of the blood. There was a moderate increase in the serum protein concentration. If one assumes that plasma proteins do not enter or

TABLE I

Changes in the volume of the blood before and after the subcutaneous administration of epinephrine

	Time after injection	Change in volume						Hemo-globin	Cells	Red blood cells	Serum proteins		Pulse	Blood pressure
		Plasma		Cells		Blood					grams	total		
	minutes	ml.	per cent	ml.	per cent	ml.	per cent	grams	per cent	per cent	grams per cent		per minute	mm. Hg
G.R.M.	Control	4330		3650		7980		15.3	45.7	45.2	6.3	268	64	110/86
Normal	8	+10	+0.2	+300	+8.2	+310	+3.9	16.0	47.6	46.8	6.7	292	78	122/82
	15	-170	-3.9	+20	+0.5	-150	-1.9	16.0	46.9	46.1			72	130/80
	22	-130	-3.0	+150	+4.1	+20	+0.3	16.0	47.5	46.7	6.8	285	78	136/76
	34	-220	-5.1	+40	+1.1	-180	-2.3		47.3	46.3			82	154/78
	46	-180	-4.2	+50	+1.4	-130	-1.6		47.1	46.3	6.6	273	78	146/78
W.O.	Control	3490		2510		6000		13.9	41.8	41.4	6.4	223	56	112/66
Normal	10	-30	-0.8	+60	+2.4	+30	+0.5		42.6	41.9	6.3	218	68	104/68
	22	-50	-1.4	+150	+6.0	+100	+1.7	14.3	43.6	43.1	6.3	217	68	114/72
	30	-120	-3.4	+120	+4.8	0	0		43.8	43.0	6.5	219	67	128/50
	41	-110	-3.2	+20	+0.8	-90	-1.5	14.4	42.8	42.2	6.4	216	70	128/52
C.H.	Control	2680		2670		5350		16.4	49.9	48.9	7.4	198	75	113/83
Normal	12	+20	+0.7	+150	+5.6	+170	+3.2		51.1	50.8	7.8	211	76	120/78
	24	-80	-3.0	+100	+3.7	+20	+0.4	16.9	51.5	49.5	7.4	192	78	120/76
	34	-60	-2.2	-30	-1.1	-90	-1.7		50.1	49.6	7.2	189	76	120/74
	47	-20	-0.7	+10	+0.4	-10	-0.2	16.8	50.2	49.6	7.1	189	82	124/72
F.G.	Control	2490		2310		4800			48.1	47.3	7.8	194	96	130/80
Normal	7	-130	-5.2	+30	+1.3	-100	-2.1		49.9	48.9	8.0	189	100	170/70
	11	-160	-6.4	-10	-0.4	-170	-3.5		49.7	49.1	7.8	182	102	165/70
	16	-170	-6.8	-20	-0.9	-190	-4.0		49.7	48.8	7.8	181	104	165/70
J.C.F.	Control	3030		2250		5280			42.6	42.0	6.9	209	64	110/78
Normal	11	-130	-4.3	+80	+3.6	-50	-0.9		44.5	43.7	7.0	203	74	118/67
	17	-130	-4.3	+110	+4.9	-20	-0.4		44.9	43.9	7.3	212	72	126/54
	27	-130	-4.3	+80	+3.6	-50	-0.9		44.5	43.5	7.0	203	70	132/54
W.P.	Control	3120		2320		5440		13.9	42.6	42.2	7.1	222	82	128/90
Rheumatoid arthritis	6	-50	-1.6	-30	-1.3	-80	-1.5	14.2	42.7	42.4	7.3	224	108	136/84
	16	-190	-6.1	+20	+0.9	-170	-3.1		44.4	43.9	7.3	214		150/80
	27	-210	-6.7	-40	-1.7	-250	-4.6	14.3	43.8	42.5	7.0	204	112	172/82
W.D.	Control	3750		3250		7000		14.1	46.4	45.5	6.1	229		136/96
Polycythemia vera	5	-90	-2.4	+240	+7.4	+150	+2.1		48.8	47.5				168/94
	11	-210	-5.6	+120	+3.7	-90	-1.3	15.0	48.8	47.5	6.4	227		148/82
	18	-220	-5.9	+50	+1.5	-170	-2.4		48.3	47.0				142/86
	20	-230	-6.1	+60	+1.8	-170	-2.4	14.7	48.4	47.1	6.3	220		138/80
J.D.	Control	4820		3790		8610		11.6	44.0	41.7	6.6	318	60	110/74
Polycythemia vera	8	-110	-2.3	+210	+5.5	+100	+1.2		45.9	43.1			68	110/74
	28	-380	-7.9	+450	+11.9	+70	+0.8		48.8	44.9	6.8	302	76	114/60
	35	-330	-6.9	+580	+15.3	+250	+2.9	12.2	49.3	44.9	6.6	296	80	118/70
	40	-380	-7.9	+450	+11.9	+70	+0.8		48.8	43.8	6.7	297	80	118/70
R.N.	Control	3160		2465		5625		13.5	43.8	42.5	6.4	202	78	121/73
Normal with splenectomy	11	-200	-6.3	-45	-1.8	-245	-4.4		45.0	43.5			72	124/70
	31	-440	-13.9	-185	-7.5	-625	-11.1	14.4	45.6	44.3	7.4	201	80	138/58
D.S.	Control	3990		3530		7520			46.9	46.0	6.6	263	68	130/90
Normal with splenectomy	12	-240	-6.0	-170	-4.8	-410	-5.5		47.3	46.3	6.9	259	90	140/90
	21	-215	-5.4	-105	-3.0	-320	-4.3		47.6	46.9	6.7	253	96	150/80
	28	-220	-5.5	-60	-1.7	-280	-3.7		47.9	47.0	6.8	256	82	154/94

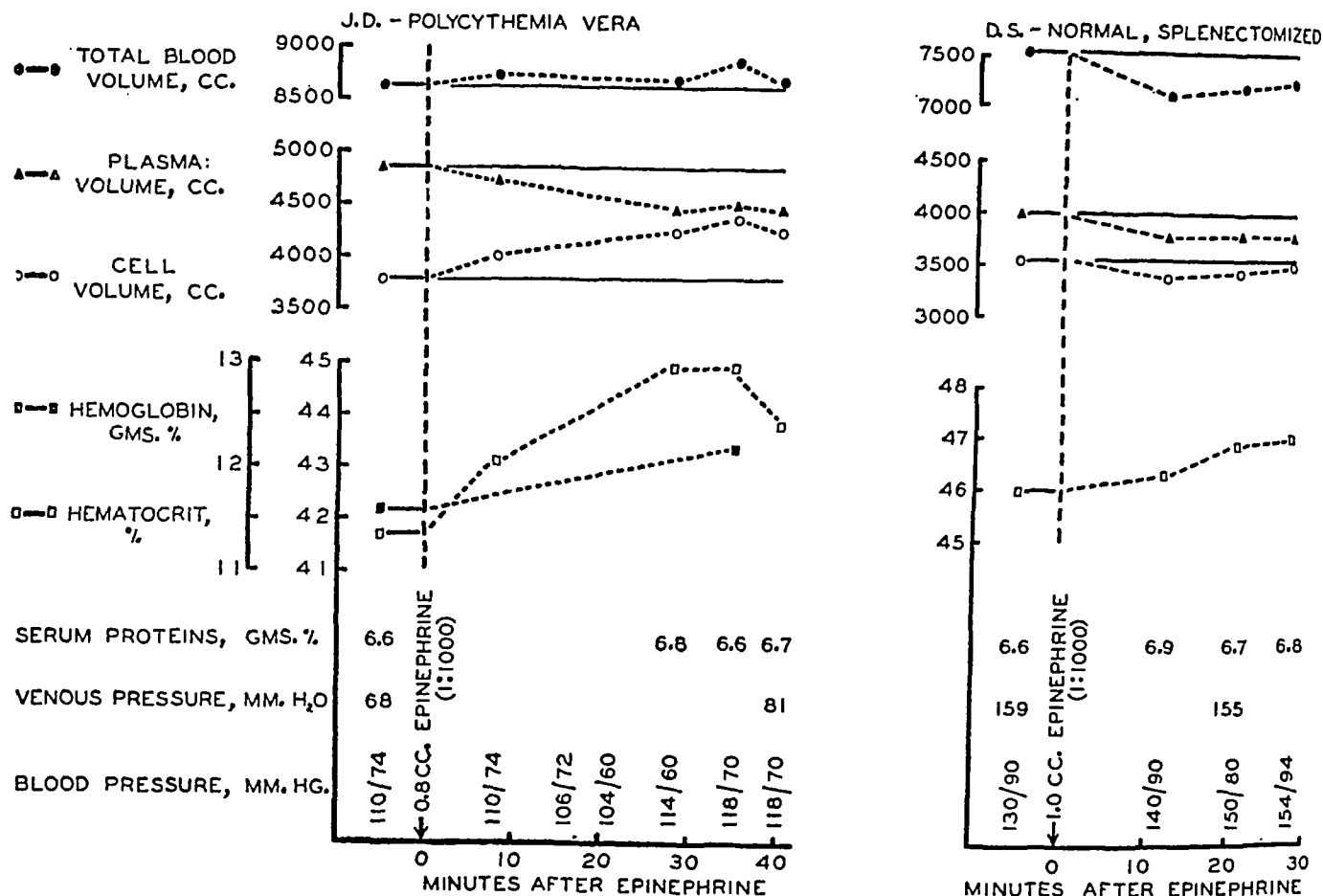


FIG. 2. CHANGES IN THE VOLUME OF THE BLOOD BEFORE AND AFTER THE ADMINISTRATION OF EPINEPHRINE IN A SUBJECT WITH POLYCYTHEMIA VERA (J. D.) AND IN A NORMAL INDIVIDUAL WHO WAS SPLENECTOMIZED (D. S.)

leave the circulating blood during this procedure, the changes in plasma volume during the experimental period can be calculated from the values of the serum proteins. The results thus obtained are in fair accord with those observed directly by the dye method. The changes in the total serum protein following the injection of epinephrine, herein demonstrated, are probably not significant. The changes are so small as to be well within the experimental limit of error of both the plasma volume and serum protein techniques.

In addition, these subjects exhibited the well known reactions of the cardiovascular system to epinephrine. The skin became pale, many complained of palpitation, headache, and a "jittery feeling". Between 24 and 46 minutes after the injection, at the height of the reaction, the level of the systolic arterial pressure was elevated in all cases; and the diastolic pressure fell in all the subjects. The pulse rate increased in all instances. The circulation time was shortened in all subjects in which it was determined. The venous pressure rose after the injection in 3 of the 5 cases.

Polycythemia vera. The changes in the blood volume following the administration of epinephrine in 2 individuals with polycythemia are illustrated in Table I and Figure 2. The initial values of the blood volume in these two cases illustrate the characteristic changes which take place when this condition is treated by venesection, as they have been repeatedly observed in this laboratory. In untreated cases of polycythemia the cell volume is markedly increased while the plasma volume remains normal, resulting in a high ratio of cells to plasma. During treatment by venesection, as the cell volume falls the plasma volume gradually increases, thereby tending to maintain a relatively high blood volume with the ratio of cells to plasma assuming a normal relationship. After repeated phlebotomies have been done, microcytosis and hypochromia develop, resembling the changes which take place in the erythrocytes in the anemias due to iron deficiency (2).

Shortly after the administration of epinephrine, there was a moderate decrease in the plasma volume which persisted for the duration of the ex-

periment. At the same time, large volumes of red cells were added to the circulating blood resulting in a slight increase in blood volume in one case (J. D.) and a slight decrease in the other (W. D.). Leukocytes were also added to the blood stream. In one case (J. D.), 35 minutes after the injections, 190 cc. of the 390 cc. increase in cell volume was due to white blood cells. In both cases at the height of the reaction the spleen was much smaller, and in one case it was no longer palpable. Coincident with these changes, the venous and arterial pressures rose in both cases, and there was concentration of hemoglobin and serum proteins.

Splenectomized subjects. The changes in the blood volume, following the injection of epinephrine in 2 subjects who were previously splenectomized, are illustrated in Table I and Figure 2. Their response was similar to that noted in the normal individuals, except that no new cells were added to the circulation. Actually the cell volume diminished after epinephrine.

DISCUSSION

It is generally known that the effect of emotional excitement or epinephrine on the peripheral blood is a rise in the number of red blood cells. The mechanism by which this erythrocytosis is brought about has not been definitely decided. There are three possible means, (a) addition of red blood cells to the circulating cell volume, (b) a diminution in the fluid constituents of the blood, or (c) a combination of these two. A perusal of the literature in reference to the volume of the blood and its components after the administration of epinephrine does not throw any light upon the question, for there is no uniformity as to the results. Following the injection of epinephrine in normal individuals, Wollheim (3) and Brandt (4), employing the dye method, observed a marked increase in both plasma and cell volumes, while Levin (5) in 80 per cent of the cases found a decrease in the plasma volume, and an increase in the blood and cell volumes in half of the cases. More consistent results were found when the carbon monoxide method was used. One group (6) observed an increase in the cell and blood volumes in all cases, while another investigator (7) found an increase in the cell volume in two-

thirds of the cases. Brednow (8) employing both methods, but neither simultaneously nor in the same subjects, noted an increase in cell volume with the carbon monoxide method and a decrease in the plasma and blood volumes when determined by the injection of dye. In the presence of splenomegaly (leukemia, polycythemia), epinephrine consistently produced an increase in the circulating cell volume when the volume was measured by either the dye or the carbon monoxide method (9, 6, 7), while in normal individuals in whom the spleen had been removed, there was no appreciable change in the cell volume (6).

The discrepancies in the results obtained by previous investigators and the rather consistent ones obtained by Ebert and Stead (10a), and by us, are due mainly to certain errors inherent in the techniques previously employed. The advantages of the present method may be summarized as follows: ample time is allowed for the mixing of dye and plasma before the experimental period is started; the determination of the plasma volume is independent of the circulatory rate; and, instead of injecting a second dose of the dye during the experimental period, changes in plasma volume following the injection of adrenalin can be calculated from the initial plasma volume, and the deviation in dye concentration from the prolongation of the disappearance slope. Recently Ebert and Stead (10b) called attention to an error in measuring by the indirect method of Gibson and Evans (11) the changes in plasma volume after exercise. This error was due to a change in the optical density of the dye-free serum. In severe or exhaustive exercise they found that as much as 40 per cent of the change in optical density attributed to an increase in dye concentration was actually due to a change in the optical density of the serum. We have repeated such experiments employing the Bausch & Lomb Spectrophotometer, and found that immediately after exhaustive exercise there is an increase in the optical density of the serum. Ten minutes after the termination of the exercise the optical density of the dye-free serum had returned to its initial value. In moderate exercise, we have not been able to detect any change in the optical density of the serum. In 6 experiments we have not been able to demonstrate a change in the optical

density of the dye-free serum after the injection of epinephrine. Since the optical density of the dye-free serum at times may increase after the injection of epinephrine, it is wise to consider these changes in the plasma volume as trends rather than precise, quantitative measurements (10a).

In all cases investigated of both normal and abnormal subjects there was a definite diminution in the plasma volume. This persisted in most instances throughout the experimental period. The rise of arterial pressure in man, after epinephrine, represents the combined effects of vasoconstriction in the skin and splanchnic areas, plus cardiac stimulation and vasodilatation in muscles, while constriction of the veins produces a rise in venous pressure. Both tend to diminish the capacity of the vascular system, and simultaneously, plasma volume.

In any discussion of the total cell volume, as measured by the dye method, it is important to realize that the volume is obtained indirectly from the plasma volume and the percentage of cells. The indications are that red cells and the plasma are not uniformly mixed within the vascular system. It has been demonstrated by Ebert and Stead (10c) that in normal subjects the cell plasma ratio varies widely throughout different parts of the circulatory system. What effect the administration of epinephrine, with its varied action on the different segments of the vascular system, may have on this relationship between cells and plasma, it is difficult to surmise. Therefore, too much reliance cannot be placed on the changes in cell volume given above. It appears from these results, that normal subjects add a few cells to the circulating blood volume; those with enlarged spleens add a somewhat larger quantity of cells to the circulation; while splenectomized individuals show a slight loss of cells following the administration of epinephrine. This difference in response can be further shown by the reaction of the blood volume to epinephrine in 2 individuals of the same body build. Both of these individuals (G. M. and D. S.) were heavy and muscular, with large surface areas and large blood volumes. One (G. M.), a normal individual, increased his cell volume after epinephrine, while the other (D. S.), a splenectomized individual, lost cells

from the circulation following the injection of this drug. These data simply show the trend of the changes in the total cell volume; they do not prove the point that erythrocytes either leave or enter the blood stream following the administration of epinephrine. Our results substantiate those of others (10a, 12, 13) which indicate that the spleen of man does not serve as an important reservoir for cells as it does in certain laboratory animals, and that under such conditions as exercise, fever, and hemorrhage, the redistribution of blood within the vascular system is a far more important compensatory reaction than contraction of the spleen.

There is a striking similarity between the effect of severe exercise on the blood volume and the response of the blood to epinephrine (1). In both circumstances there is a hemoconcentration due in part to a shift of a moderate amount of fluid, poor in protein, from the vascular system to the interstitial compartment, and in part to the accession of a small quantity of new cells from the blood depots. The former is produced by increased capillary pressure, while the latter is mediated through the sympathetic-adrenin mechanism.

SUMMARY AND CONCLUSIONS

Measurements were made at rest of the volume of the blood and its components, and variations in the volumes were followed after the subcutaneous injection of 1 cc. of epinephrine (1-1000). Further observations included measurements of the blood hemoglobin and viscosity, serum proteins, venous and arterial pressures, velocity of the blood, and pulse rate. These observations lead to the following conclusions:

1. In normal individuals, following the administration of epinephrine, there is a prompt and definite decrease in the plasma volume, which persists in most cases for at least 45 minutes. In the majority of cases there is a slight increase in the cell volume. These alterations are associated with an increase in blood hemoglobin and viscosity and serum proteins. Following the administration of the drug, the systolic pressure increased while the diastolic pressure fell slightly.

2. In individuals who have polycythemia vera with splenomegaly, epinephrine causes a definite

decrease in the plasma volume, a moderate increase in cell volume with little change in the total volume.

3. After the injection of epinephrine into 2 individuals whose spleens had been removed, there was a decrease in both blood and plasma volumes, accompanied by a slight decrease in the cell volume.

4. The effects of severe exercise and of epinephrine on the components of the blood volume are similar.

BIBLIOGRAPHY

1. Kaltreider, N. L., and Meneely, G. R., The effect of exercise on the volume of the blood. *J. Clin. Invest.*, 1940, 19, 627.
2. Stephens, D. J., and Kaltreider, N. L., The therapeutic use of venesection in polycythemia. *Ann. Int. Med.*, 1937, 10, 1565.
3. Wollheim, E., Die zirkulierende Blutmenge und ihre Bedeutung für Kompensation und Dekompensation des Kreislaufs. *Ztschr. f. klin. Med.*, 1931, 116, 269.
4. Brandt, F., Die Abhängigkeit des Venendruckes von der Grösse der zirkulierenden Blutmenge, zugleich ein Beitrag zur Frage seiner klinischen Bedeutung. *Ztschr. f. klin. Med.*, 1931, 116, 398.
5. Levin, E., La acción de la adrenalina sobre el volumen de la sangre circulante. *Rev. méd. del Rosario*, 1935, 25, 255.
6. Yang, C. S., and Chang, H. C., Effect of adrenalin on circulating blood volume in individuals with normal and enlarged spleens and after splenectomy. *Chinese J. Physiol.*, 1930, 4, 21.
7. Hitzenger, K., and Tuchfeld, F., Über den Einfluss des Adrenalins auf die zirkulierende Blutmenge. *Klin. Wchnschr.*, 1929, 8, 1208.
8. Brednow, W., Beeinflussung der zirkulierenden Blutmenge und der Blutverteilung durch physikalische und pharmakologische Massnahmen. Einfluss von Adrenalin, Pituitrin und Histamin. *Ztschr. f. d. ges. exper. Med.*, 1931, 78, 177.
9. Grunke, W., Einfluss des Adrenalins auf die kreisende Plasma—und Blutmenge bei chronischer Milzvergrösserung. *Ztschr. f. klin. Med.*, 1934, 127, 542.
10. (a) Ebert, R. V., and Stead, E. A., Jr., Demonstration that in normal man no reserves of blood are mobilized by exercise, epinephrine, and hemorrhage. *Am. J. Med. Sci.*, 1941, 201, 655.
(b) Ebert, R. V., and Stead, E. A., Jr., An error in measuring changes in plasma volume after exercise. *Proc. Soc. Exper. Biol. and Med.*, 1941, 46, 139.
(c) Ebert, R. V., and Stead, E. A., Jr., Demonstration that the cell plasma ratio of blood contained in minute vessels is lower than that of venous blood. *J. Clin. Invest.*, 1941, 20, 317.
11. Gibson, J. G., 2nd, and Evans, W. A., Jr., Clinical studies on the blood volume. I. Clinical application of a method employing the azo dye "Evans Blue" and spectrophotometer. *J. Clin. Invest.*, 1937, 16, 301.
12. Dill, D. B., Talbott, J. H., and Edwards, H. T., Studies in muscular activity. VI. Response of several individuals to a fixed task. *J. Physiol.*, 1930, 69, 267.
13. Keys, A., and Taylor, H., The behavior of the plasma colloids in recovery from brief severe work and the question as to the permeability of the capillaries to proteins. *J. Biol. Chem.*, 1935, 109, 55.

COMPLEMENT ACTIVITY IN PNEUMONIA¹

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In the evaluation of the immunity mechanism in man during pneumococcus pneumonia, complement studies have received little attention. Publications concerning the level of antibody in relation to recovery from pneumonia have been summarized up to 1939 by Heffron (1), and other studies have been published since that time (2 to 6). The few studies regarding complement activity in pneumonia which have been published present conflicting conclusions. Dick (7) studied 4 pneumonia patients and 2 controls and reported that complement activity increased during pneumonia and returned to normal after crisis. Veil and Buchholz (8) stated that complement activity did not decrease during pneumonia but presented no data. Robertson, Sia, and Cornwell (9) reported without specific data that "little evidence was obtained to show that the activating effect of fresh animal (including human) serum undergoes any significant alteration during the disease." Taplin (10) noted that 6 patients who failed to respond to adequate and specific serum therapy were found to be deficient in serum complement. Dingle (11) studied 73 patients with many diseases, 9 of whom had pneumonia. Only 2 of the 73 patients had diminished complement activity, and both of these were pneumonia patients.

It has been demonstrated *in vitro* by Robertson, Sia, and Cornwell (9) that the addition of fresh normal serum increased the pneumococcal-promoting action of Type I antipneumococcus serum, and Ward and Enders (12) have reported that the opsonic effect of Type II type-specific antipneumococcus antibody is enhanced by the addition of complement, even though complement, in the absence of antibody, has no effect. Also, complement activity in the blood of normal individuals, as reported in the medical literature (9, 12 to 16, 17 to 21), is remarkably constant within a com-

paratively narrow range. These findings, particularly those of Taplin, suggested the need for further study of complement activity in pneumonia.

Accordingly, complement activity was studied in the blood serum of 75 patients admitted to the Albany Hospital from February 1940 to June 1941 for the treatment of pneumococcus pneumonia. This report presents the observations of that study with particular reference to outcome, pneumococcus type, bacteremia, serum administration, drug administration and serum sickness, although not every case was included in each series.

The diagnosis of pneumonia was confirmed in every case by an x-ray photograph of the chest and by demonstration of type-specific pneumococci on direct examination of the sputum (Neufeld).

METHODS

Blood specimens were collected, allowed to clot, centrifuged, and the sera pipetted off in the usual fashion. Sterile dry equipment was used throughout. The specimens were numbered and allowed to stand at refrigerator temperature overnight. They were then transferred, without any accompanying information other than the name of the patient and the date of bleeding, to the laboratory of Dr. Frank Maltaner² at the Division of Laboratories and Research of the New York State Department of Health. Specimens were kept at refrigerator temperature from the time of separation of the serum up to the time of testing, with the exception of the time required for transfer of the specimens to the laboratory. The distance between the hospital and the laboratory is but a few hundred yards, and it is believed that no appreciable effect on complement activity would be caused by the time required for that transfer.

The titrations were usually performed within forty-eight hours of the time that the blood was collected, although a few specimens were titrated as long as seventy-two hours after bleeding. Studies will be re-

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² We wish to acknowledge the cooperation and advice extended to us by Dr. Maltaner, and express our thanks for the technical assistance of Miss Loretta Dugan who performed the laboratory tests.

with normal complement activity on admission, as compared with 2 deaths among the 12 cases with low complement activity on admission (Table II). It is therefore evident that complement activity at the time of admission to the hospital could not be used as an index of prognosis in this series of patients. However, it is important to note that complement activity was lower prior to death than at the time of admission to the hospital in 6 of the 7 patients on whom more than one determination was performed (Table IV). The results of the final tests indicated low complement activity in 6 of the 9 patients from whom specimens were collected within seventy-two hours of death.

All specimens of low activity at the time of admission to the hospital were obtained from patients with either Type I, III or VII pneumococcus infections (Table V). The sera of all of the 31 patients infected with types other than I, III and VII were within the normal range of complement activity at the time of admission to the hospital. The distribution of Type I patients was striking, since 9 of the 12 cases with low complement activity on admission were Type I cases, whereas only 12 of the 59 cases with normal complement activity on admission were found to be Type I infections.

Blood cultures were done on all patients at the time of admission to the hospital and no correla-

TABLE III

Complement activity of sera of pneumonia patients with low complement activity at time of admission to hospital and complement activity of same patients following recovery*

Case number	Volume of serum*		Percentage increase in volume* at time of admission
	During pneumonia at time of admission	Following recovery	
	ml.	ml.	
2	0.0069	0.0044	+ 56.8
59	0.0069	Died	
40	0.0083	Died	
64	0.0087	0.0036	+ 141.7
12	0.0077	0.0030	+ 156.7
66	0.0100	0.0038	+ 163.2
55	0.0110	0.0030	+ 266.7
39	0.0230	0.0031	+ 641.9
52	0.1000	0.0030	+3233.3
21	0.2000	0.0051	+3821.6
47	>0.2000†	0.0045	>+4344.4
15	>0.2000†	0.0031	>+6351.6

* See footnote on Table I.

† Less than 50 per cent hemolysis with 0.2 ml. undiluted serum, the largest amount tested.

TABLE IV

Complement activity of sera of pneumonia patients at time of admission to hospital and on final test before death*

Case number	At time of admission to hospital		Final test before death	
	Volume of serum*	Interval to death	Volume of serum*	Interval to death
	ml.	days	ml.	days
72	0.0028	1	0.0041	0
27†	0.0040	1	0.0040	1
45	0.0049	2	>0.2000‡	2
40†	0.0083	2	0.0083	2
5	0.0060	3	0.0085	1
57	0.0030	5	0.0204§	3
33	0.0039	7	0.0030	3
60	0.0051	22	0.1778	0
17†	0.0036	30	0.0036	30
59	0.0069	33	0.0202	2

* See footnote on Table I.

† Only one test performed.

‡ Two specimens taken on the same day, one before and one following serum administration. The final specimen was taken two hours after serum administration (Table VI).

§ Specimen taken one day following serum administration (Table VI).

|| See footnote on Table III.

tion could be found between the occurrence of bacteremia and the presence of low complement activity, since bacteremia occurred in 16 of the 59 patients with normal complement activity, and in 4 of the 12 patients with low complement activity.

Age and sex had no effect on complement activity in this group of patients, either on admission to the hospital or after recovery from the disease. These findings with respect to age are in agreement with those of Gunn (14) who reported that there were no differences in complement activity in normal individuals according to age.

The frequency of low complement activity among alcoholic patients at the time of admission to the hospital (3 in 20 patients) was similar to that among non-alcoholic patients (9 in 51 patients).

The occurrence of a diminished complement activity in the blood serum of certain pneumonia patients at the time of admission to the hospital is striking when compared with the marked uniformity of complement activity in normal individuals, but the significance of this change is not clear. A study of antigen, antibody and complement relationships in the blood of pneumonia patients may yield important information regarding the mechanism of recovery or death from

TABLE I

*Distribution of apparently healthy individuals according to complement activity**

Volume of serum*	Apparently healthy individuals
ml.	
0.0028-0.0029.....	1
0.0030-0.0039.....	4
0.0040-0.0049.....	23
0.0050-0.0059.....	19
0.0060-0.0067.....	7
Total.....	54

* Complement activity is inversely related to the volume of serum required to produce 50 per cent hemolysis in a standardized system. Therefore, an increase in volume indicates a decrease in complement activity, and a decrease in volume indicates an increase in complement activity.

ported elsewhere showing that the time interval between bleeding and testing was not a factor in the changes in complement activity discussed in this paper.

The titrations were performed according to the technic of Wadsworth, Maltaner and Maltaner (22), with adjustment of the dilutions to correct for the fact that complement activity of human serum is approximately half that of guinea pig serum (16, 23). The advantages of this method, the end point of which is the amount of serum required to effect 50 per cent hemolysis in a standardized system, as compared with those dependent upon the choice of the one tube in which hemolysis begins or ends, have been reported by Wadsworth, Maltaner and Maltaner (22). The chemical method of Heidelberger, recently published (24, 25), could not be used in a study of this nature because of the large amount of human serum required for that test.

Complement activity is reported in this study in terms of the volume of blood serum in milliliters required to produce 50 per cent hemolysis. Since the volume required is inversely related to complement activity, the larger the number of milliliters indicated, the lower the complement activity in the specimen of the blood serum, and vice versa.

Range of normal complement activity

The normal range of complement activity of human blood as determined by this method confirms the marked constancy of complement activity in normal individuals as measured by other methods (9, 12 to 16, 17 to 21).

Blood specimens were obtained from 54 apparently healthy individuals and the largest amount of serum required to effect 50 per cent hemolysis in this group was 0.0067 ml. (Table I). This will be considered as the lower limit of the range

of normal complement activity for purposes of discussion in this paper and volumes of 0.0068 ml. or greater will be referred to in this study as indices of low complement activity.

Complement activity during pneumonia

Of the 71 pneumonia patients from whom blood specimens were collected on admission to the hospital, 12, or 16.9 per cent, were found to have low complement activity varying from 0.0069 ml. to complete failure of hemolysis in the largest amount of serum tested, *i.e.*, 0.2 ml. of undiluted serum. However, the specimens collected from these patients following recovery were found to have normal complement activity with a range of 0.0030 to 0.0051 ml. The percentage increase in complement activity on recovery was largest among those with the lowest complement activity at the time of admission to the hospital (Table III). There was no apparent trend in either direction among the 51 recovered cases with normal complement activity on admission to the hospital.

The case fatality rate among the 71 patients tested on admission to the hospital was 14.1 per cent. There were 8 deaths among the 59 patients

TABLE II

Distribution of pneumonia patients at time of admission to hospital and following recovery from pneumonia, according to complement activity and outcome*

Volume of serum*	Pneumonia patients		
	At time of admission		Following recovery
	Recovered	Died	
ml.			
Normal			
0.0023-0.0029....	3	1	3
0.0030-0.0039....	18	3	27
0.0040-0.0049....	17	2	16
0.0050-0.0059....	11	1	14
0.0060-0.0067....	2	1	1
Total...	51	8	61
Low			
0.0068-0.0069....	1	1	
0.0070-0.0079....	1		
0.0080-0.0089....	1	1	
0.0090-0.0099....			
0.0100-0.2000....	7†		
Total...	10	2	
Grand total.	61	10	61

* See footnote on Table I.

† Specimens from two of these showed less than 50 per cent hemolysis with 0.2 ml. undiluted serum, the largest amount of serum tested.

with normal complement activity on admission, as compared with 2 deaths among the 12 cases with low complement activity on admission (Table II). It is therefore evident that complement activity at the time of admission to the hospital could not be used as an index of prognosis in this series of patients. However, it is important to note that complement activity was lower prior to death than at the time of admission to the hospital in 6 of the 7 patients on whom more than one determination was performed (Table IV). The results of the final tests indicated low complement activity in 6 of the 9 patients from whom specimens were collected within seventy-two hours of death.

All specimens of low activity at the time of admission to the hospital were obtained from patients with either Type I, III or VII pneumococcus infections (Table V). The sera of all of the 31 patients infected with types other than I, III and VII were within the normal range of complement activity at the time of admission to the hospital. The distribution of Type I patients was striking, since 9 of the 12 cases with low complement activity on admission were Type I cases, whereas only 12 of the 59 cases with normal complement activity on admission were found to be Type I infections.

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52	0.1000	0.0030	+3233.3
21	0.2000	0.0051	+3821.6
47	>0.2000†	0.0045	>+4344.4
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	ml.	days	ml.	days
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27†	0.0040	1	0.0040	1
45	0.0049	2	>0.2000‡	2
40†	0.0083	2	0.0083	2
5	0.0060	3	0.0085	1
57	0.0030	5	0.0204§	3
33	0.0039	7	0.0030	3
60	0.0051	22	0.1778	0
17†	0.0036	30	0.0036	30
59	0.0069	33	0.0202	2

* See footnote on Table I.

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The occurrence of a diminished complement activity in the blood serum of certain pneumonia patients at the time of admission to the hospital is striking when compared with the marked uniformity of complement activity in normal individuals, but the significance of this change is not clear. A study of antigen, antibody and complement relationships in the blood of pneumonia patients may yield important information regarding the mechanism of recovery or death from

pneumonia. It would also be desirable to study complement activity in other diseases, especially during the days immediately preceding death, in order to discover whether the decrease in complement activity is a specific effect of the pneumonia or a general biological phenomenon which becomes manifest in many diseases in the period prior to death.

Complement activity after intravenous serotherapy and chemotherapy

The complement activities of specimens of blood serum taken immediately preceding and following intravenous administration of antipneumococcus horse serum, antipneumococcus rabbit serum, sodium sulfathiazole, and sodium sulfadiazine were compared.

There were decreases in complement activity after serum administration in 16 of 19 patients who received either horse or rabbit antipneumococcus serum (Table VI). These results are in marked contrast to those observed in patients who received approximately equal sized intravenous injections of 5 per cent solutions of sodium sulfathiazole or sodium sulfadiazine in distilled water. Seven of the 14 patients who received these drugs demonstrated decreases in complement activity (Table VII), but the degrees of change were less marked than among those patients who received serum (Table VI).

In this series of patients, there were no differences between the changes occurring after the injection of antipneumococcus horse and rabbit serum (Table VI). This differs from the results obtained *in vitro* by Zinsser and Parker (26), and confirmed with purified specific polysaccharides by

TABLE V

Distribution of pneumonia patients at time of admission to hospital, according to complement activity and predominant type of pneumococcus

Pneumococcus type	Pneumonia patients		
	Total	Normal complement activity	Low complement activity
I.....	21	12	9
III.....	14	12	2
VII.....	5	4	1
All other.....	31	31	
Total.....	71	59	12

TABLE VI

Complement activity of sera of pneumonia patients immediately preceding and immediately following intravenous antipneumococcus serum administration*

Case number	Serum	Volume of serum*		Percentage increase or decrease in volume* following serum administration
		Preceding serum administration	Following serum administration	
		ml.	ml.	
36	Rabbit	0.0977	0.0091	— 90.7
42	Rabbit	0.0047	0.0042	— 10.6
58	Rabbit	0.0035	0.0035	No change
60	Horse	0.0051	0.0054	+ 5.9
48	Rabbit	0.0033	0.0035	+ 6.1
43	Horse	0.0065	0.0070	+ 7.7
54	Horse	0.0056	0.0061	+ 8.9
49	Rabbit	0.0057	0.0064	+ 12.3
63	Horse	0.0041	0.0050	+ 22.0
69	Rabbit	0.0055	0.0070	+ 27.3
68	Rabbit	0.0048	0.0062	+ 29.2
56	Rabbit	0.0056	0.0081	+ 44.6
75	Horse	0.0036	0.0056	+ 55.6
64	Horse	0.0087	0.0148	+ 70.1
66	Horse	0.0100	0.0186	+ 86.0
52	Horse	0.1000	>0.2000†	>+ 100.0
55	Rabbit	0.0110	0.0881	+ 700.9
45	Rabbit	0.0049	>0.2000†	>+3981.6
57	Rabbit	0.0030	0.1445	+4716.7

* See footnote on Table I.

† See footnote on Table III.

Goodner and Horsfall (27), showing that antipneumococcus rabbit serum "under proper conditions" after union with pneumococcus antigen will fix complement, whereas antipneumococcus horse serum under the same circumstances will not do so.

The decreases in complement activity following serum administration may possibly explain the occasional failure of huge amounts of antipneumococcus serum to control the disease in certain patients. Such failures occurred in the era prior to chemotherapy. This explanation is a likely one considering the experimental evidence of the enhancement of the opsonic and bactericidal effects of antipneumococcus serum by the addition of complement (9, 12). Moreover, the tendency of complement activity to decrease during the period prior to death (Table IV) may be one of the reasons for the relative failure of serotherapy when administered late in the course of the disease.

Complement activity during serum sickness

Serum sickness, for purposes of this study, is defined as a delayed reaction occurring at least one day following the administration of antipneumococcus serum and consisting of any one or a

TABLE VII

Complement activity of sera of pneumonia patients immediately preceding and immediately following intravenous drug administration*

Case number	Drug	Volume of serum*		Percentage increase or decrease in volume* following drug administration
		Preceding drug administration	Following drug administration	
		ml.	ml.	
74	S. Sd.	0.0033	0.0029	-12.1
56	S. Sth.	0.0040	0.0038	-5.0
57	S. Sd.	0.0030	0.0029	-3.3
73	S. Sd.	0.0043	0.0042	-2.3
75	S. Sd.	0.0039	0.0039	No change
70	S. Sd.	0.0031	0.0031	No change
60	S. Sd.	0.0051	0.0051	No change
53	S. Sth.	0.0065	0.0068	+4.6
59	S. Sd.	0.0069	0.0075	+8.7
65	S. Sth.	0.0063	0.0070	+11.1
62	S. Sd.	0.0048	0.0054	+12.5
67	S. Sth.	0.0052	0.0063	+21.2
71	S. Sd.	0.0033	0.0041	+24.2
61	S. Sth.	0.0033	0.0041	+24.2

* See footnote on Table I.

S. Sth. = Sodium Sulfathiazole.

S. Sd. = Sodium Sulfadiazine.

combination of the following symptoms: urticaria, arthritis, and lymphadenopathy. These are frequently accompanied by fever, but in patients recovering from pneumonia it is not feasible to consider fever alone as diagnostic of serum sickness. Therefore, individuals presenting fever alone are not included in the serum sickness study. The severity of the disease varied from a few wheals lasting for a few hours to severe urticaria, arthritis, and lymphadenopathy lasting for six days. Only patients who recovered from pneumonia prior to the onset of serum sickness are included in Table VIII.

The blood specimens used in determining complement activity during serum sickness were taken during the first day of that disease and the results obtained were compared with those of blood specimens taken before serum sickness, during pneumonia at the time of admission to the hospital, and after serum sickness, following recovery from that disease.

One-half of the patients studied during serum sickness showed marked decreases in complement activity at that time (Table VIII). These changes could not be correlated with the nature or the severity of the symptoms presented, but further studies are being made in an attempt to discover

why striking decreases in complement activity occurred in only half of these patients.

These results in patients with serum sickness are not inconsistent with the evidence of the literature: the decrease in complement activity in 4 animals with experimental serum sickness, as reported by Miura (28), and in the single human case, as reported by Francioni (29).

CONCLUSIONS

The blood serum of apparently healthy individuals had remarkably constant hemolytic complement activity within a comparatively narrow range.

At the time of admission to the hospital, the blood serum of one-sixth of the pneumonia patients studied had low complement activity. No correlation could be found between complement activity and age, sex, bacteremia or alcoholism.

No specimens of low complement activity were found on recovery from pneumonia.

Complement activity could not be used as an index of prognosis at the time of admission to the hospital in this series of pneumonia patients, but there was a tendency for complement activity to diminish in the period prior to death.

There was an unusual incidence of Type I infection among patients with low complement activity at the time of admission to the hospital.

TABLE VIII

Complement activity of sera of pneumonia patients preceding, during, and following serum sickness*

Case number	Volume of serum*		
	Preceding serum sickness	During serum sickness	Following serum sickness
	ml.	ml.	ml.
22	0.0032	0.0025	0.0039
14	0.0037	0.0026	0.0055
10	0.0046	0.0040	0.0036
36	0.0977	0.0042	Not done
21	0.2000	0.0049	0.0051
18	0.0034	0.0051	Not done
75	0.0039	0.0068	0.0038
68	0.0048	0.0068	0.0042
6	Not done	0.0120	0.0074
26	0.0041	0.0123	0.0053
30	0.0037	0.0200	0.0046
64	0.0087	0.0200	0.0036
25	0.0049	0.0400	0.0048
20	0.0054	0.1122	Not done
63	0.0041	>0.2000†	0.0030
66	0.0100	>0.2000†	0.0038

* See footnote on Table I.

† See footnote on Table III.

Complement activity of specimens of blood collected immediately following intravenous administration of antipneumococcus horse and rabbit serum was, in most cases, lower than the activity of specimens obtained from the same patients prior to serum administration. In contrast, the changes which occurred following the injection of sodium sulfathiazole and sodium sulfadiazine were not remarkable.

In one-half of the individuals studied, complement activity was lower during serum sickness than before or after serum sickness.

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BIBLIOGRAPHY

1. Heffron, R., *Pneumonia with Special Reference to Pneumococcus Lobar Pneumonia*. The Commonwealth Fund, New York, 1939, p. 863.
2. Finland, M., Spring, W. C., Jr., and Lowell, F. C., Immunological studies on patients with pneumococcal pneumonia treated with sulfapyridine. *J. Clin. Invest.*, 1940, 19, 179.
3. Kneeland, Y., Jr., and Mulliken, B., Antibody formation in cases of lobar pneumonia treated with sulfapyridine. *J. Clin. Invest.*, 1940, 19, 307.
4. Fox, W. W., Rosi, R., and Winters, W. L., Sabin agglutination test as control of sulfapyridine treatment of pneumonia. *Am. J. M. Sc.*, 1940, 200, 78.
5. Fox, W. W., Rosi, R., and Winters, W. L., Sabin agglutination test and polysaccharide skin test (Francis) as indices of recovery in pneumonia. *Am. J. M. Sc.*, 1940, 200, 649.
6. Kneeland, Y., Jr., and Mulliken, B., Antibody formation in cases of lobar pneumonia treated with sulfathiazole. *J. Clin. Invest.*, 1940, 19, 735.
7. Dick, G. F., On the development of proteolytic ferments in the blood during pneumonia. *J. Infect. Dis.*, 1912, 10, 383.
8. Veil, W. H., and Buchholz, B., Der Komplementschwund im Blute. *Klin. Wchnschr.*, 1932, 11, 2019.
9. Robertson, O. H., Sia, R. H. P., and Cornwell, M. A., Activating effect of fresh normal serum on pneumococcal-promoting action of antipneumococcus serum, Type I. *J. Immunol.*, 1930, 19, 429.
10. Taplin, G., Serum treatment of pneumococcal pneumonia. *J. A. M. A.*, 1940, 115, 1676.
11. Dingle, J. H., Personal communication.
12. Ward, H. K., and Enders, J. F., Analysis of opsonic and tropic action of normal and immune sera based on experiments with pneumococcus. *J. Exper. Med.*, 1933, 57, 527.
13. Neisser, E., and Doering, H., Zur Kenntnis der haemolytischen Eigenschaften des menschlichen Serums. *Berl. klin. Wchnschr.*, 1901, 38, 593.
14. Gunn, W. C., The variation in the amount of complement in the blood in some acute infectious diseases and its relation to the clinical features. *J. Path. and Bact.*, 1914, 19, 155.
15. Goldner, M., Untersuchungen über das Komplement im Serum bei Leberkranken. *Deutsche med. Wchnschr.*, 1929, 55, 390.
16. Bauer, R., and Weiss, I., Über den Komplementgehalt des menschlichen Serums. *Med. Klin.*, 1930, 26, 1635.
17. Deisler, K., and Montgomery, L. G., Studies on complement function in serum of man. *Proc. Staff Meet.*, Mayo Clin., 1934, 9, 157.
18. Paul, B., and PeLy, M., Über die Abnahme des Blutkomplementgehaltes bei allergischen Krankheiten. *Klin. Wchnschr.*, 1935, 14, 163.
19. Bernstein, R. E., Maingard, J. F., and Osborn, T. W. B., Note on haemolytic complement in normal Europeans and Bantu. *South African J. M. Sc.*, 1935, 1, 63.
20. Kellett, C. E., Complement titre in acute nephritis, with special reference to causation by reversed anaphylaxis. *Lancet*, 1936, 2, 1262.
21. Thomson, S., Arnott, W. M., and Matthew, G. D., Blood complement in acute glomerulonephritis and toxæmia of pregnancy. *Lancet*, 1939, 2, 734.
22. Wadsworth, A., Maltaner, E., and Maltaner, F., Quantitative determination of fixation of complement by immune serum and antigen. *J. Immunol.*, 1931, 21, 313.
23. Maltaner, F., Unpublished observations.
24. Heidelberger, M., Quantitative, absolute method for estimation of complement (alexin). *Science*, 1940, 92, 534.
25. Heidelberger, M., Quantitative chemical studies on complement or alexin. I. A method. *J. Exper. Med.*, 1941, 73, 681.
26. Zinsser, H., and Parker, J. T., Bacterial hypersusceptibility. *J. Exper. Med.*, 1923, 37, 275.
27. Goodner, K., and Horsfall, F. L., Jr., Complement-fixation reaction with pneumococcus capsular polysaccharide. *J. Exper. Med.*, 1936, 64, 201.
28. Miura, T., Untersuchungen über die experimentelle Serumkrankheit. *Tr. Soc. path. jap.*, 1940, 30, 378.
29. Francioni, C., La diminuzione del complemento nella malattia da siero. *Riv. di clin. pediat.*, 1908, 6, 321.

THE COAGULASE TEST FOR STAPHYLOCOCCI AND ITS CORRELATION WITH THE RESISTANCE OF THE ORGANISMS TO THE BACTERICIDAL ACTION OF HUMAN BLOOD^{1,2}

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Advances in the study of the staphylococcus and infections caused by this micro-organism include the development of biological and serological methods for differentiating various strains of staphylococci. Numerous investigators have concluded that the coagulase reaction is one of the most practical and simple methods for differentiating pathogenic from non-pathogenic strains (1 to 7). We have arrived at similar conclusions, but it should be emphasized that the use of the terms, "pathogenic", and "non-pathogenic", express only a relative relationship. As will be pointed out, non-pathogenic strains may in rare instances, cause serious and even fatal infections.

The purpose of this report is to correlate the coagulase reaction with the resistance of staphylococci to the bactericidal action of human blood. This work is an outgrowth of a previous study in which it was shown that pathogenic strains of staphylococci were highly resistant to the antibacterial action of human bloods from healthy controls and from those recovering from severe staphylococcal infections (8). As far as we can ascertain from a review of the literature, no comprehensive reports are available concerning the relationship of coagulase production to the growth of staphylococci in human blood. In this connection, Thompson and Khorazo (9) stated that Type A strains of staphylococci grew better in human defibrinated blood than non-Type A strains.

METHODS OF STUDY

Source of strains. A total of 70 strains were studied, all isolated from human beings. Thirty-two strains were obtained from an equal number

of patients having severe infections such as bacteremia, osteomyelitis, and bacterial endocarditis. Fifteen strains were sent to us by Dr. G. H. Chapman of New York City in answer to a request for human strains which he considered to be non-pathogenic. Ten strains of *S. albus* were obtained from superficial lesions and human urine. A strain of *S. albus* was cultured from the venous bloods of each of two patients with a bacterial endocarditis. Eleven strains of *S. albus* were grown from the hair and skin of normal human beings.

Cultures were grown on veal-agar slants, pH 7.8, kept in a refrigerator and transplants made every 3 weeks. Continuous transfers were made over a period of 3 months to 3 years, depending upon the time when the original culture was obtained. During the last year of study, the majority of the cultures were maintained under oil on veal-agar slants and transplants made every 6 months.

Coagulase test. The test was performed by transferring one loopful of organisms from a 24-hour culture on an agar slant to 0.5 ml. of citrated human plasma and incubating the plasma in a water bath at 37° C. The pressure of coagulum at the end of 3 or 18 hours indicated a positive test.

Bactericidal test. Eighteen-hour broth cultures were used. The broth as described by Lyons (10) consisted of veal infusion (Difco), peptone (2 per cent), glucose (0.05 per cent), and NaCl (1 per cent). Fresh, defibrinated blood from individuals free of demonstrable infections was used. Ten-fold dilutions of the broth cultures were made up to the 10⁷ dilution and 0.05 ml. of each dilution was added to each of several small, pyrex glass tubes containing 0.5 ml. of defibrinated blood. The approximate number of organisms in each of the dilutions was determined by preparing pour-

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² Presented at the Annual Meeting of the Central Society for Clinical Research, Chicago, November 7, 1941.

TABLE I

Correlation between coagulase and bactericidal test for staphylococci isolated from patients with severe infections

Strain number	Pigment	Coagulase test	Bactericidal test*							
			Dilution of organisms							Number of organisms per 0.05 ml. 10 ⁷ dilution
			10 ¹	10 ²	10 ³	10 ⁴	10 ⁵	10 ⁶	10 ⁷	
1	Yellow	Positive	+	+	+	+	+	+	+	202
2	Yellow	Positive	+	+	+	+	+	+	+	331
3	Yellow	Positive	+	+	+	+	+	+	+	26
4	Orange	Positive	+	+	+	+	+	+	+	250
5	Yellow	Positive	+	+	+	+	+	+	+	56
6	Orange	Positive	+	+	+	+	+	+	+	500
7	Yellow	Positive	+	+	+	+	+	+	+	500
8	Orange	Positive	+	+	+	+	+	+	+	389
9	Orange	Positive	+	+	+	+	+	+	+	3
10	Yellow	Positive	+	+	+	+	+	+	+	160
11	Gold	Positive	+	+	+	+	+	+	+	102
12	White	Positive	+	+	+	+	+	+	+	12
13	Gold	Positive	+	+	+	+	+	+	+	215
14	Orange	Positive	+	+	+	+	+	+	+	83
15	Yellow	Positive	+	+	+	+	+	+	+	344
16	Yellow	Positive	+	+	+	+	+	+	+	190
17	White	Positive	+	+	+	+	+	+	+	185
18	White	Positive	+	+	+	+	+	+	+	64
104	Orange	Positive	+	+	+	0	0	0	0	200

* + = Growth.

plates with 0.05 ml. of the 10⁶ and 10⁷ dilutions. The tubes were sealed in a gas-oxygen flame and rotated for 24 hours in a box in an incubator at 37° C. At the end of this time, the tubes remained for another 24 hours in the incubator. They were then opened and the contents of each tube cultured on an agar plate to determine the presence of viable organisms.

RESULTS

All 32 strains isolated from patients having severe infections were coagulase-positive and resisted the bactericidal action of normal defibrinated blood with but one exception. Representative results are presented in Table I. It is to be noted that strains 12, 17, and 18 grew out as white colonies, and on this basis would be classified as *S. albus* cultures. In this connection, experience with strain 18 is of interest. It was isolated from the blood stream of a patient who had a fulminating and fatal infection. The presence of *S. albus* in the culture brought up the possibility of its being a contaminant. Subsequent cultures of blood yielded the same type of colonies. Repeated coagulase tests performed with different cultures of this strain gave positive results which classified the culture as belonging to the pathogenic group. After many subcultures, this strain still produces only white colonies and is resistant

to the bactericidal action of whole blood. *S. albus* strains 12 and 17 were obtained from severe osteomyelitic lesions. Strain 104 was the only coagulase-positive strain that did not resist the killing effect of blood. Repeated tests with this strain, using samples of blood from several individuals, showed that a significant number of organisms were killed in every instance. However, the strain was highly pathogenic because of a lethal toxin that it produced. A laboratory worker accidentally inoculated with a broth culture of this strain expired 52 hours later. The clinical course and autopsy findings were similar to those observed by Kellaway and his associates in the Bundaberg disaster of 1928 (11).

Several veal-agar transplants were made with the 15 non-pathogenic *S. albus* cultures sent to us in 1937 by Dr. George A. Chapman. These were stated to be coagulase-negative strains. In our hands, 2 of these strains were found to be coagulase-positive, and resisted the bactericidal action of blood. The significance of these findings will be discussed shortly. The remaining cultures were all coagulase-negative, and a marked killing effect of the blood was observed for all of the strains.

Table II includes the results with a group of 12 strains of *S. albus* isolated from persons with superficial lesions and low-grade infections of the urinary tract, and from 2 patients with bacterial endocarditis. Strain 157 was coagulase-positive and no killing effect was exhibited by the whole

TABLE II

Correlation between coagulase and bactericidal tests for *S. Albus* isolated from human beings

Strain number	Coagulase test	Bactericidal test*							
		Dilution of organisms							Number of organisms per 0.05 ml. 10 ⁷ dilution
		10 ¹	10 ²	10 ³	10 ⁴	10 ⁵	10 ⁶	10 ⁷	
150	Negative	+	0	0	0	0	0	0	53
151	Negative	+	+	0	0	0	0	0	600
152	Negative	0	0	0	0	0	0	0	15
153	Negative	+	+	0	0	0	0	0	69
154	Negative	+	0	0	0	0	0	0	131
155	Negative	+	+	+	+	0	0	0	229
156	Negative	+	+	0	0	0	0	0	656
157	Positive	+	+	+	+	+	+	+	100
158	Negative	+	+	+	0	0	0	0	23
159	Negative	+	+	0	0	0	0	0	50
160	Negative	+	+	+	0	0	0	0	251
161	Negative	+	+	0	0	0	0	0	22

* + = Growth.

TABLE III

Correlation between coagulase and bactericidal tests at intervals of three years for S. Albus strains

Strain number	1938										1941											
	Pigment production	Coagulase test	Bactericidal test*								Organisms 10 ⁷ dilution	Pigment production	Coagulase test	Bactericidal test*								Organisms 10 ⁷ dilution
			Dilution of organisms											Dilution of organisms								
			10 ¹	10 ²	10 ³	10 ⁴	10 ⁵	10 ⁶	10 ⁷	10 ¹				10 ²	10 ³	10 ⁴	10 ⁵	10 ⁶	10 ⁷			
6508	White	Negative	+	+	+	0	0	0	0	475	Yellow	Positive	+	+	+	+	+	+	+	27		
6744	White	Negative	+	+	+	0	0	0	0	1021	Yellow	Positive	+	+	+	+	+	+	+	23		
6707	White	Positive	+	+	+	+	+	+	+	135	Yellow	Positive	+	+	+	+	+	+	+	21		
6715	White	Negative	+	+	0	0	0	0	0	116	White	Negative	+	+	0	0	0	0	0	68		
6476	White	Negative	+	+	0	0	0	0	0	500	White	Negative	+	+	0	0	0	0	0	43		
6756	White	Negative	+	+	+	0	0	0	0	311	White	Negative	+	+	+	+	0	0	0	31		
6773	White	Negative	+	+	0	0	0	0	0	30	White	Negative	+	+	0	0	0	0	0	4		
6780	White	Positive	+	+	+	+	+	+	+	135	White	Negative	+	+	0	0	0	0	0	44		
155	White	Negative	+	+	+	+	0	0	0	229	Yellow	Positive	+	+	+	+	+	+	+	23		
157	White	Positive	+	+	+	+	+	+	+	100	White	Positive	+	+	+	+	+	+	+	43		

* + = Growth.

blood when this strain was investigated. This culture was obtained from the urine of a patient only mildly ill. In contrast, organisms from all of the 11 remaining strains were killed in large numbers. Strains 153 and 161 were isolated from the blood streams of 2 patients who had subacute bacterial endocarditis. Repeated blood cultures revealed the same species of organism to be present. Organisms from both strains grew out as white colonies; they showed a negative coagulase test; and they were killed in large numbers by the whole blood of the patients and of normal individuals. The blood streams of both individuals were sterile for long periods of time following the use of sulfonamide compounds. Cardiac failure supervened in both patients and death resulted. Post-mortem studies were carried out and clumps of cocci were present at the bases of the vegetations on the mitral valves of both cases.

Table III includes the results of the coagulase and bactericidal tests performed in 1938 and in 1941 with the same strains. During the intervening period of three years, numerous transplants had been made on veal-agar. All the strains, except 155 and 157, were procured from Dr. G. H. Chapman. The significant feature of these observations is that four of the strains (6508, 6744, 6780, and 155) showed a reversal in the results of the coagulase tests, and coincident with this, a change in their resistance to the killing power of blood. A possible explanation for these changes will be presented shortly.

DISCUSSION

While the foregoing data show a remarkably close correlation between the production of coagulase by staphylococci and the resistance of the organisms to the bactericidal action of defibrinated human blood, we do not wish to imply that coagulase, *per se*, is the factor responsible for the difference in the antibacterial action of blood against coagulase-positive and coagulase-negative strains. It is not known what effect this substance has upon the killing power of whole blood.

It was noted that some strains after many transplants exhibited a reversal of coagulase production and of their resistance to the bactericidal action of blood. It is not unlikely that the explanation for these biological differences is one of bacterial dissociation. It is well known that avirulent *S. albus* colonies may occur as variants of *S. aureus* strains (12, 13). These non-pathogenic *S. albus* variants have been shown to be coagulase-negative by Pinner and Voldrich (13). Blair also observed that a small percentage of coagulase-positive strains will lose their ability to coagulate plasma over a period of several months (14).

SUMMARY

1. The coagulase test is the simplest and most reliable method for differentiating pathogenic from non-pathogenic strains of staphylococci.
2. Coagulase-positive strains of staphylococci resist the bactericidal action of human defibrinated

blood; whereas, coagulase-negative strains are killed in large numbers, with only two exceptions in a study of 70 strains.

3. The terms "pathogenic" and "non-pathogenic", based on the results of the coagulase test, are relative since coagulase-negative strains on rare occasions may result in fatal infections. Two instances of subacute bacterial endocarditis are recorded to illustrate this.

4. Repeated subcultures of coagulase-positive strains may result in coagulase-negative strains. The reverse of this also occurs. Both phenomena are probably explained on the basis of bacterial dissociation.

BIBLIOGRAPHY

1. Fisher, A. M., The plasma-coagulating properties of staphylococci. *Bull. Johns Hopkins Hosp.*, 1936, 59, 393.
2. Cruickshank, R., Staphylocoagulase. *J. Path. and Bact.*, 1937, 45, 295.
3. Cowan, S. T., The classification of staphylococci by precipitation and biological reactions. *J. Path. and Bact.*, 1938, 46, 31.
4. Blair, J. E., The pathogenic staphylococci. *Bact. Rev.*, 1939, 3, 97.
5. Fisk, A., Technic of coagulase test for staphylococci. *Brit. J. Exper. Path.*, 1940, 21, 311.
6. Fairbrother, R. W., Coagulase production as a criterion for the classification of the staphylococci. *J. Path. and Bact.*, 1940, 50, 83.
7. Chapman, G. H., Berens, C., and Stiles, M. H., The coagulation of plasma by staphylococci. *J. Bact.*, 1941, 41, 431.
8. Spink, W. W., and Paine, J. R., The bactericidal power of blood from patients and normal controls for staphylococci. *J. Immunol.*, 1940, 38, 383.
9. Thompson, R., and Kharazo, D., Differential growth of the antigenic types of staphylococci in human blood. *J. Bact.*, 1938, 35, 51.
10. Lyons, C., Antibacterial immunity to staphylococcus pyogenes. *Brit. J. Exper. Path.*, 1937, 18, 411.
11. Kellaway, C. H., MacCallum, P., and Terbutt, A. H., Report of the Royal Commission of enquiry into the fatalities at Bundaberg, 1928.
12. Hoffstadt, R. E., and Youmans, G. P., Staphylococcus aureus: Dissociation and its relation to infection and to immunity. *J. Infect. Dis.*, 1932, 51, 216.
13. Pinner, M., and Voldrich, M., Derivation of staphylococcus *albus*, *citreus*, and *roseus* from staphylococcus aureus. *J. Infect. Dis.*, 1932, 50, 185.
14. Blair, J. E., The stability of biological and biochemical properties of staphylococci. *J. Bact.*, 1938, 35, 52.

CALCULATION OF HEAT PRODUCTION FROM INSENSIBLE LOSS OF WEIGHT¹

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In a previous communication, Newburgh and his associates (1) proposed a method for calculating 24-hour heat production from I. W.² rather than from I. L.,² since variations in respiratory quotient cause the relationship of I. W. to I. L. to vary from 85 to 100 per cent. However, in reviewing the literature recently, we were impressed with two characteristics of Levine and Marples' (2) prediction curve which they obtained when they plotted I. L. against heat production in 196 cases. These data were obtained by means of the Russel Sage calorimeter or with the respiration chamber at the New York Nursery and Child's Hospital. The two characteristics were, first, the almost constant percentage of heat removed by vaporization of water throughout the whole course of the line and, second, that the origin of the line is not far from zero.

Consequently, we decided to re-examine our own material to determine how closely heat production can be predicted from I. L. rather than from I. W.

The smoothed curve of Levine and Marples is reproduced in Figure 1. We have plotted 34 of our own experiments with 10 normal adults³ on this diagram. They consist of simultaneous determinations of I. L. and heat production for 24-hour periods in the respiration chamber. Further experiments on 11 additional subjects³ were also included. Here we have used for the I. L. the average of many consecutive 24-hour periods. The average 24-hour heat production in this series was determined by feeding a carefully weighed constant diet that closely approached maintenance

as a result of preliminary trials. The 24-hour heat production was taken to be the energy of this diet, corrected for small changes in weight which occasionally occurred. In these experiments, the periods always exceeded one month. Further, it is important to note that these 11 subjects were leading their usual lives except that they were asked to avoid the feeling of chilliness or warmth by adjustment of clothing. Several single periods had to be excluded because these conditions could not be obtained.

Inspection of Figure 1 reveals that only 4 of our 45 points fall outside of the ± 15 per cent limits, even though the line has been extended from about 2,000 to 3,500 calories. The average percentage of heat lost by vaporization for these subjects was 24.7 per cent. The percentage of heat lost by vaporization is not quite constant throughout Levine and Marples' line, as pointed out by Heller (5) who calculated that the percentage at the lower end was 27.6 and at the upper end, 24.2.

Since it seems probable that a constant percentage of heat is removed by vaporization of water throughout the whole range of environmental temperatures at which chilling and sweating can be prevented, we decided to draw a line which begins at zero I. L. and zero heat and whose slope throughout represents a loss of 24.5 per cent of total heat removed by vaporization. Since it is desired to express this relationship in terms of I. L. rather than as I. W., the latter needs to be converted to the former.

Consideration of Levine and Marples' prediction, where approximately 25 per cent of the heat was removed by vaporization of water, shows that close to 94 per cent of I. L. is water vapor. Further study of the data obtained in this laboratory, from normal individuals existing on usual mixed diets, gives a value of 93 per cent.

Hence our prediction line for normal human beings existing on usual mixed diets has also been

¹ The expense of this study was defrayed in part by a grant from the Horace H. and Mary A. Rackham School of Graduate Studies.

² I. W. is an abbreviation for the weight of water vaporized from skin and lungs. I. L. is an abbreviation for the insensible loss of weight.

³ These details of the methods and the biological data may be found in earlier publications from this laboratory (1, 3, 4).

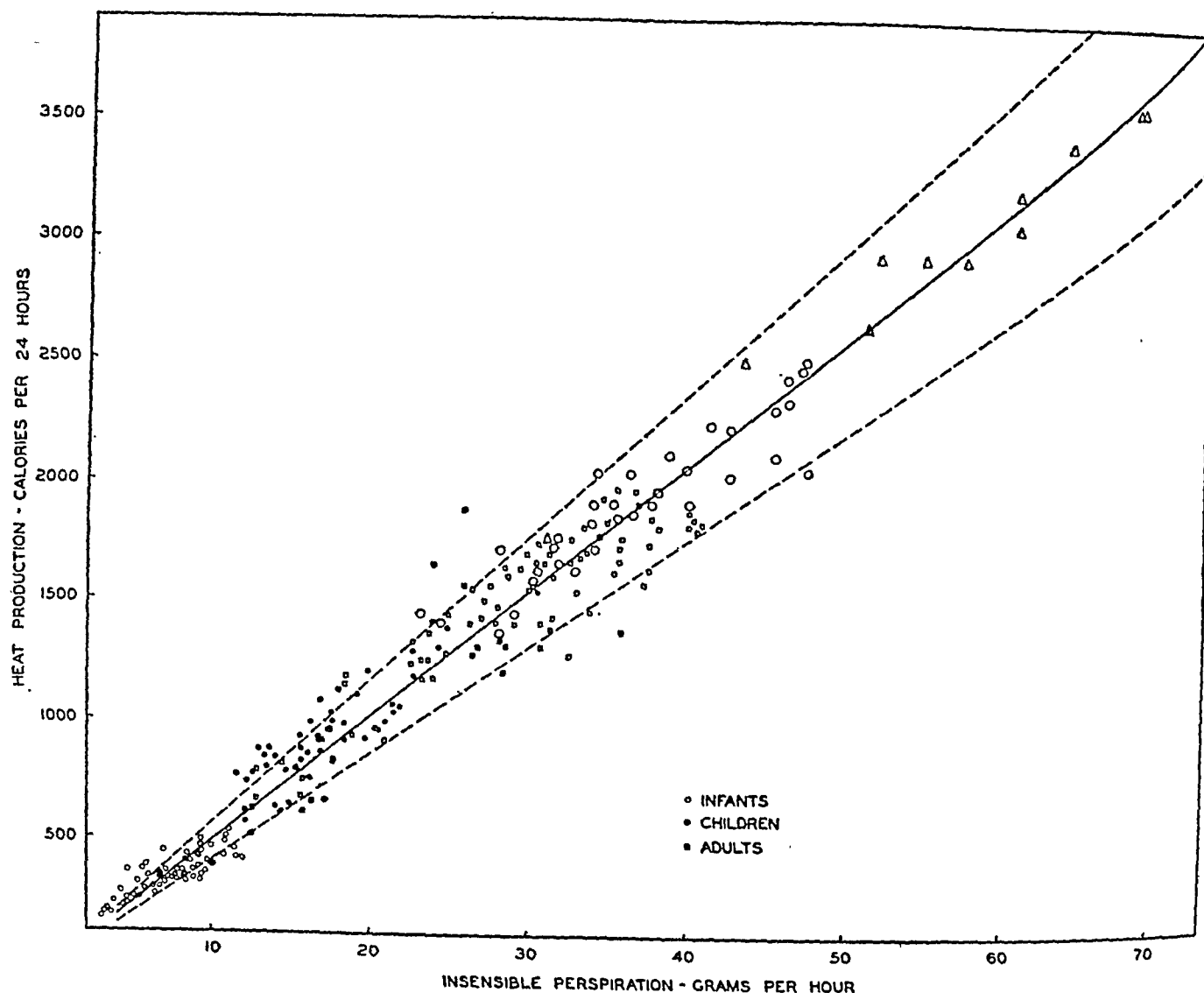


FIG. 1. REPRODUCTION OF LEVINE AND MARPLES (2) SMOOTHED CURVE ON WHICH WE HAVE PLACED OUR OWN DATA. Levine and Marples values are represented by \circ for infants, \bullet for children and \square for adults. Our own data are represented by \odot for simultaneous determination of I.L. and indirect calorimetry for 24 hours; and by Δ for I. L. and heat production calculated from diet and change in weight.

constructed so that I. W. is 93.5 per cent of I. L. (Figure 2, line A).

In Figure 2 also we have reproduced lines from several publications for purposes of comparison with our own prediction line. It will be seen that the smoothed curve of Levine and Marples (line B) is almost identical with ours (line A). It departs slightly at the lower end and this may well be due to the difficulty of dealing with infants. The data gathered by Ginandes and Topper (6), using children 4 to 15 years of age, are represented by line C. The I. L. was determined for periods of 2 to 3 hours and the heat production, by the Benedict-Roth apparatus, before or after the weighings. Their line is significantly below ours. This suggests that

the I. L.s are somewhat high, due to restlessness on the part of children expected to remain perfectly quiet for so long a time. Had the heat production been determined in the middle of the weighings, higher values might have been recorded. On the other hand, Levine and Marples were able to obtain simultaneous records of I. L. and heat production.

Line D represents the prediction proposed by Benedict and Root (7); Jores' (8) data follow the same line. Here the percentage of heat lost by vaporization of water is not constant. It is 21.9 per cent at the lower end and 32.5 at the upper end. Furthermore, if one projects the curve downward, at 493 calories the I. L. would be zero. Perhaps this situation is attributable to

the use of emaciated diabetics for low heat productions and of hyperthyroids for large heat productions. Furthermore, the I. L. was determined from 10 to 15 minute periods. Wiley and Newburgh (9) found that short periods gave irregular results even when trained normal subjects were employed. Jores followed the procedure proposed by Benedict and Root and his series also included a large amount of pathological material. The same criticism applies to the data of Laszlo and Schurmeyer (10) and to those of Heller (5).

However, Heller and Schwartz (11) analyzed 1936 cases studied in the respiration calorimeter taken from the early experiments of Atwater, followed by those of Benedict and of DuBois. They dealt with subjects in the basal state, with others after food, and with a third group during work. Heat productions beyond 4,000 calories were excluded. They derived an equation for the lines representing each of the three groups. When I. L. is plotted against heat there is a marked difference in the slope of each of the

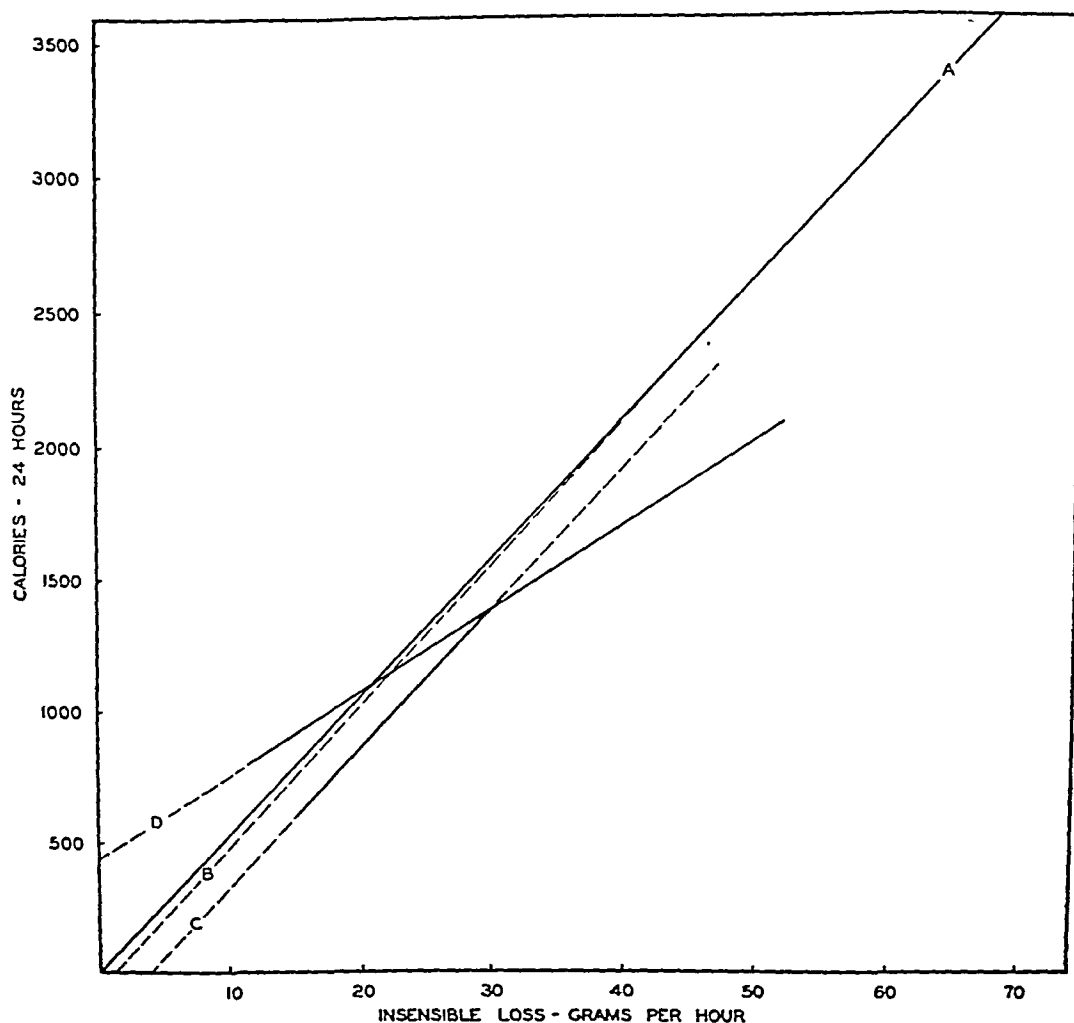


FIG. 2

Line A was constructed so that it represents a loss of 24.5 per cent of the heat by vaporization of water and so that $\frac{I.W.}{I.L.} \times 100 = 93.5$. Broken line B, Levine and Marples' (2) smoothed curve. Line C, Ginandes and Topper (6). Line D, Benedict and Root (7).

TABLE I

Comparison of heat production calculated from I.W. and from I.L. with that determined by indirect calorimetry for 24 hour periods

Date	Subject	I.L.	Heat production					Heat lost by vaporization
			Indirect calorimetry	From I.L.				
				Calories M.M. ^a	Difference columns 4 and 5	Calories I.L. × 53 ^a	Difference columns 4 and 7	
		grams per hour			per cent		per cent	per cent of total
1935								
January 14	B.deV.	31.5	1772	1634	-7.7	1670	-5.3	24.2
February 24	B.deV.	30.9	1729	1696	-1.9	1640	-5.2	24.5
March 24	B.deV.	34.	2059	1827	-11.3	1804	-12.4	23.2
March 31	B.deV.	36.	2065	1928	-6.5	1908	-7.6	22.6
November 24	B.deV.	34.	1929	1755	-9.0	1804	-6.5	23.7
November 28	B.deV.	33.6	1828	1725	-5.6	1780	-2.6	23.3
December 8	B.deV.	37.	1925	2035	+5.4	1960	+1.8	25.4
December 23	B.deV.	41.	2242	2134	-4.7	2172	-3.1	23.7
1936								
February 2	B.deV.	39.7	2079	2054	-1.2	2105	+1.3	24.6
February 4	B.deV.	45.7	2430	2531	+4.2	2420	-0.4	24.4
February 28	McQ.	31.5	1567	1743	+11.2	1670	+6.6	27.7
March 21	McQ.	28.	1725	1537	-10.9	1485	-13.9	23.4
1935								
April 22	W.M.	38.5	2124	2124	±0.0	2040	-3.9	26.0
December 26	W.M.	46.5	2486	2596	+4.2	2465	-0.8	26.0
1936								
January 2	W.M.	45.9	2346	2434	+3.8	2432	+3.7	26.7
January 12	W.M.	46.8	2501	2493	-0.3	2480	-0.8	25.0
1935								
October 10	J.S.	39.7	1935	2196	+13.5	2103	+8.7	27.6
November 6	J.S.	37.8	1987	2059	+3.1	2002	+0.8	26.6
November 12	J.S.	36.2	1889	1876	-0.7	1909	+1.1	25.1
November 19	J.S.	35.	1924	1808	-6.0	1855	-7.5	23.7
November 21	R.L.G.	45.2	2110	2387	+13.1	2398	+13.6	28.5
December 5	R.L.G.	45.	2215	2344	+5.8	2382	+7.5	27.2
December 12	R.L.G.	42.3	2054	2358	+14.8	2240	+9.0	28.8
December 19	R.L.G.	42.3	2229	2353	+5.6	2240	+0.5	26.4
1936								
January 30	R.L.G.	47.4	2070	2461	+18.9	2510	+21.2	29.8
March 4	H.A.	34.9	1852	1844	-0.4	1850	-0.1	25.1
February 9	L.W.P.	33.7	1743	1853	+6.6	1786	+2.5	25.5
March 1	L.W.P.	30.3	1656	1658	+0.1	1593	-3.8	24.8
January 15	M.B.	28.	1343	1539	+14.6	1485	+10.6	27.3
January 22	M.B.	24.5	1415	1348	-4.7	1298	-8.3	22.7
January 19	O.M.	30.1	1594	1486	-6.0	1595	+0.0	23.4
January 24	O.M.	32.4	1654	1656	+0.1	1716	+3.7	25.7
February 18	D.R.	29.	1441	1586	+10.8	1536	+6.6	27.0
February 26	D.R.	23.	1447	1252	-13.4	1219	-15.7	21.2

rial dealt with by Levine and Marples consisted of observations lasting only several hours. While we are convinced that periods of 10 to 20 minutes give irregular results, it is probable that the method is applicable to periods much shorter than 24 hours.

As in all methods, it is essential to guard against certain errors. In this case it is especially im-

^a Fifty-three is the factor obtained from line A, Figure 3, by dividing 24-hour calories by the corresponding hourly I. L. at any point.

portant to train the subject to maintain himself in a comfortable state without feeling cool or warm. Since the sweating mechanism becomes active at only a few degrees above the environmental temperature at which most individuals are comfortable, it is important not to allow the room temperature to rise above 72° F. In order to know that the subject is trained, it is desirable to insist upon uniform activity during the training period. When under these circumstances consistent I. L.s are obtained, one has evidence that

TABLE II

Comparison between heat calculated from I. L. and heat production determined from the calories of the diet corrected for change in weight

Subject	I.L.	Heat production			Difference	
		From diet	I.L. X53	I.L. X57 ¹¹	Columns 3 and 4	Columns 3 and 5
	<i>grams per hour</i>				<i>per cent</i>	<i>per cent</i>
F.D.J. ¹⁰	51.5	2950	2730	2936	-7.5	-0.5
G.G. ¹⁰	30.5	1766	1615	1739	-8.5	-1.5
T.M. ¹⁰	43.1	2500	2285	2457	-8.6	-1.7
R.S.	57.1	2960	3030		+2.4	
B.deV.	50.8	2664	2690		+1.0	
F.H.W.	54.3	2947	2878		-2.3	
F.H.W.	60.6	3082	3212		+4.2	
A.W.	68.4	3570	3625		+1.5	
R.L.G.	68.0	3550	3640		+2.5	
M.P.	63.5	3425	3365		-1.8	
M.W.	60.4	3210	3200		-0.3	

the technique is satisfactory. It appears to be true that the tendency to lose 24.5 per cent of the heat by vaporization of water does not exhibit the constancy that is shown by internal temperature. Hence a single period may not follow the rule and consequently reliable prediction of heat from I. L. can be made only when the average I. L. of a series of determinations is used as the basis of calculation.

We have dealt primarily with normal adults. Whether the method is valuable in the study of disease remains to be decided by future work.

SUMMARY

Twenty-four hour heat production can be satis-

¹⁰ Diabetic subjects on low carbohydrate diets.

¹¹ Fifty-seven is the factor (I. W. = I. L.), used for low carbohydrate plans.

factorily predicted from the insensible loss of weight. Factors have been derived for use in accordance with the type of diet fed.

BIBLIOGRAPHY

1. Newburgh, L. H., Johnston, M. W., Lashmet, F. H., and Sheldon, J. M., Further experiences with the measurement of heat production from insensible loss of weight. *J. Nutrition*, 1937, 13, 203.
2. Levine, S. Z., and Marples, E., The insensible perspiration in infancy and in childhood. *Am. J. Dis. Child.*, 1930, 40, 269.
3. Newburgh, L. H., Wiley, F. H., and Lashmet, F. H., A method for the determination of heat production over long periods of time. *J. Clin. Invest.*, 1931, 10, 703.
4. Wiley, F. H., and Newburgh, L. H., The doubtful nature of "Luxuskonsumption." *J. Clin. Invest.*, 1931, 10, 733.
5. Heller, H., Die quantitativen Beziehungen zwischen Perspiration insensibilis und Energieumsatz. *Ztschr. f. d. ges. exper. Med.*, 1932, 83, 128.
6. Ginandes, G. J., and Topper, A., Statistical correlation of insensible perspiration and basal metabolism. *Am. J. Dis. Child.*, 1937, 53, 705.
7. Benedict, F. G., and Root, H., Insensible perspiration: Its relation to human physiology and pathology. *Arch. Int. Med.*, 1926, 38, 1.
8. Jores, A., Perspiratio insensibilis. *Ztschr. f. d. ges. exper. Med.*, 1930, 71, 170.
9. Wiley, F. H., and Newburgh, L. H., The relationship between the environment and the basal insensible loss of weight. *J. Clin. Invest.*, 1931, 10, 689.
10. Laszlo, D., and Schurmeyer, A., Perspiratio insensibilis und Grundumsatz. *Ztschr. f. klin. Med.*, 1931, 116, 22.
11. Heller, H., and Schwarz, A., Extrarenale Wasserausscheidung und Stoffwechsel. *Ztschr. f. d. ges. exper. Med.*, 1930, 71, 416.

THE EFFECTS ON THE CARDIOVASCULAR SYSTEM OF FLUIDS ADMINISTERED INTRAVENOUSLY IN MAN. IV. THE LUNG VOLUME AND PULMONARY DYNAMICS

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Earlier studies from this laboratory (1, 2, 3) have shown that the intravenous administration of 500 to 1500 cc. of fluids, at rates ranging from 9 to 125 cc. per minute, results in appreciable increases in plasma and blood volumes. Significant changes in cardiovascular dynamics did not occur at rates of injection under 20 cc. per minute; administration of fluids intravenously at faster rates resulted in rises in venous pressure and cardiac output, slight increases in pulse rate and pulse pressure, and, in some experiments, acceleration of the velocity of blood flow. Acceleration of blood flow usually occurred (2) when 500 to 600 cc. of fluid were given intravenously, at rates of 20 to 30 cc. per minute; infusions given in larger volume or at more rapid rates caused unexpectedly small increases, or even transitory decreases, in the velocity of blood flow. It was therefore believed that transitory increase in the amount of blood in the lungs may have occurred under these circumstances. Studies of respiratory dynamics were made (2) in several patients who received 400 to 1000 cc. of fluid intravenously, at rates of between 11 and 36 cc. per minute; no changes were found in respiratory rate or minute volume, tidal air, or vital capacity. However, it was apparent that additional studies of respiratory dynamics should be made after larger infusions, given more rapidly; accordingly, measurements of respiratory dynamics and of the various subdivisions of the lung volume have been made in subjects receiving 1800 cc. of fluid intravenously, at rates of between 39 and 185 cc. per minute.

MATERIAL AND METHODS

Six subjects, ranging in age from 18 to 44 years, were used in this study; one (case 5) was female. None had evidence of any abnormality of the cardiovascular or respiratory systems. All received 1800 cc. of isotonic saline solution intravenously, at rates ranging between 39 and 185 cc. per minute. The subdivisions of the lung

volume were estimated by the method of Christie (4), slightly modified (5), the respiratory dynamics being measured at the same time. Duplicate studies of these pulmonary functions and estimations of the hematocrit and plasma protein were made before the infusion and again at the end of injection. The first of the measurements of lung volume, after infusion, was completed as the infusion ended or within three minutes thereafter. Immediately following this, the reserve and complementary airs were measured and then the second of the post-infusion estimations of the lung volume was made. The hematocrit and plasma protein concentration were measured as in previous studies (1, 2, 3). Calculations of the changes in blood volume were based on methods previously described (1).

OBSERVATIONS

The *functional residual* and *residual airs* before and after the injection of fluids showed no differences outside the limits of error of the method used (Table I).

The changes in the other components of the total lung volume and in the total lung volume itself were in most instances only slight and frequently well within the limits of error of the method. However, there was a consistency in the changes which demonstrated a tendency toward a decrease (Table I), as is evident from the following resumé. The *reserve air* was diminished in all instances after injection, the decreases ranging between 30 and 240 cc., or 2.5 and 26.8 per cent of the original volumes. The average decrease was 98 cc. or 10.9 per cent. The *complemental air* was diminished in all instances after injection, the decreases ranging between 40 and 360 cc., or 1.0 and 12.3 per cent of the original volumes. The average decrease was 198 cc. or 6.6 per cent. The *vital capacity* was diminished in all instances after injection, the decreases ranging between 105 and 600 cc., or 2.1 to 14.3 per cent of the original volumes. The average decrease was 296 cc. or 7.4 per cent. The *lung*

TABLE I
Effects of intravenous infusions on blood and lung volumes and respiratory dynamics

Case	Rate of infusion	Venous pressure	Hematocrit	Serum protein	Plasma volume	Blood volume	Functional residual air	Residual air	Reserve air	Complemental air	Vital capacity	Total capacity	Tidal air	Respirations	Respiratory volume	Oxygen consumption	Remarks
	cc. per minute	cm.	per cent	grams per cent	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	per minute	liters per minute	cc. per minute	
1	39	7.6 11.6	43.6 38.0	6.76 5.69	3270 3890	5800 6280	3660 3630	2550 2680	1110 990	3925 3885	5035 4875	7585 7515	675 655	11 11.5	7.43 7.53	305 279	Before After
2	54.6	5.0 10.1	42.0 37.6	6.38 5.21	2580 3160	4450 5070	2185 2230	1430 1555	755 675	2775 2580	3530 3255	4960 4810	785 615	9 14	7.07 8.61	204 182	Before After
3	68	7.2 13.2	46.2 42.0	7.46 6.59	3150 3560	5850 6140	3940 3800	2760 2650	1180 1150	3925 3850	5105 5000	7805 7650	475 435	15 16	7.10 6.98	286 310	Before After
4	92	3.0 10.0	44.6	7.55 6.42	2630 3090	4750	2360 2140	1465 1485	895 655	3295 2935	4190 3590	5635 5095	435 395	16 19.5	6.96 7.70	245 242	Before After
5	180	9.0 13.5	41.2 33.8	6.11 4.56	2470 3310	4200 5000	1710 1815	1195 1340	515 475	1945 1705	2460 2180	3675 3520	515 480	14.5 15	7.47 7.14	239 224	Before After
6	185	7.2 12.3	44.2 40.0	6.86 5.83	3570 4200	6400 7000	2595 2495	1445 1425	1150 1070	4160 3885	5310 4955	6755 6380	435 485	18 17.5	7.83 8.49	319 337	Before After

volume was diminished in all instances, the decreases ranging between 70 and 540 cc., or 0.9 and 9.6 per cent of the original volumes. The average decrease was 241 cc. or 4.2 per cent.

The respiratory minute volume increased significantly after the infusion in three of the six experiments, being essentially unchanged in the other three. The increases in respiratory minute volume were due in two instances to increases in respiratory rate and in one, to augmented tidal air volume. In five of the six experiments, the volume of the tidal air was somewhat diminished.

The above described changes in the pulmonary dynamics and subdivisions of the total lung volume were still detectable 40 minutes after the end of infusion.

DISCUSSION

Earlier studies (2) have shown that intravenous infusions of 400 to 1000 cc. of fluid, at rates between 11 and 36 cc. per minute, resulted in no change in vital capacity, respiratory rate, or minute volume. Richards *et al.* (6) observed slight decreases in vital capacity in normal subjects after infusions of 1500 cc. of isotonic sodium chloride solution at 50 cc. per minute; these decreases were never greater than 8 per cent of the initial vital capacity. In the present study, the three subjects

who received 1800 cc. of fluid, at rates of 39 to 55 cc. per minute, showed changes within this limit; two of the three receiving the fluid at 90 to 185 cc. per minute showed only slightly greater decreases in vital capacity (Table I). The two components of the vital capacity, *i.e.*, the reserve and complemental airs, showed approximately the same degree of change following infusion. Since the residual air did not change, it is clear that the slight decreases observed in total lung volume were due to diminution of the vital capacity. In general, the changes in the total lung volume and its various subdivisions were too small to be considered physiologically significant.

Congestive failure results in a relative increase in residual and functional residual air volumes as compared to total lung volume (7). In the present study changes in the ratios between functional residual and residual air volumes and the total lung volume were small and not consistently in the same direction (Table II). It is clear, therefore, that large intravenous infusions, given at rapid rates in normal subjects, do not produce the typical pulmonary signs of congestive failure. None of the subjects developed dyspnea, orthopnea, cough or other symptoms, nor were râles audible over the chest. Studies in animals corroborate this conclusion. Warthen (8) gave dogs

weighing 6.7 to 26.5 kgm., 900 to 2600 cc. of fluids intravenously at rates of 29 to 169 cc. per minute without producing pulmonary edema. One animal, weighing 9.4 kgm., developed pulmonary edema after receiving 3700 cc. in 35 minutes. Cohnheim and Lichtheim (9) found that rabbits might develop edema of the lungs after receiving intravenous infusions equivalent to approximately half their body weight; in dogs, pulmonary edema only rarely developed after the intravenous administration of fluids equivalent to as much as 90 per cent of their body weight. The slowing of blood flow previously noted (2) and the slight decreases in vital capacity observed in the present study and by Richards *et al.* (6) after large, rapidly administered intravenous infusions are to be ascribed to a minor degree of pulmonary vasodilatation, associated with increased total blood volume resulting from the infusions.

Evidences of speed shock (10) were not observed in these or previous studies in which fluid was given intravenously at rapid rates. It is not to be concluded, however, that the rapid administration of large intravenous infusions is likewise without deleterious effect in individuals who are not normal. Richards *et al.* (6) reported a marked decrease in vital capacity, dyspnea, and the appearance of râles in the lungs, after such infusions in cardiac patients; patients with various forms of pulmonary disease also exhibited marked diminution in the vital capacity (6, 11). In our own clinical experience, patients with severe uremic acidosis develop signs and symptoms of pulmonary congestion and edema consistently with intravenous infusions of relatively small amounts of fluid given at rates as low as 10 cc. per minute or slower. Experiments in animals

afford similar data. Kraus (12), Brunn (13) and Farber (14) have described increased susceptibility to pulmonary edema in animals following bilateral vagotomy. Farber found that whereas intact rabbits showed no pulmonary edema after intravenous infusions of 250 to 400 cc. of isotonic sodium chloride solution at rates of 30 to 40 cc. per minute, bilaterally vagotomized animals developed severe edema of the lungs after receiving smaller volumes at slower rates. The fact that changes in pulmonary dynamics and lung volume following rapid intravenous injections of large volumes of fluid in normal subjects were at most only slight, in no way alters the clinical concept that when it is necessary to administer fluids intravenously in patients with a tendency toward pulmonary congestion and edema because of cardiac, pulmonary, central nervous system, or renal disease, these infusions should be given at slower rates and with caution.

SUMMARY AND CONCLUSIONS

1. Studies of the effect of the injection of fluids intravenously on the subdivisions of the lung volume and on the respiratory dynamics have been made in six normal subjects.

2. Injection intravenously of 1800 cc. of isotonic sodium chloride solution, at rates of 39 to 185 cc. per minute, in these normal subjects caused no change in residual air, and only slight decreases in the vital capacity, its components, the reserve and complemental airs, and in the total lung volume. The respiratory minute volume showed no consistent change, although the tidal air was usually decreased. All the changes in pulmonary function found after intravenous infusions in these normal subjects were insignificant.

3. The slight decreases in vital capacity, its components, and the total lung volume, after these massive intravenous infusions at rapid rates in these normal subjects, are interpreted as due to slight pulmonary vasodilatation associated with temporarily increased blood volume.

4. The fact that changes in pulmonary dynamics and lung volume, following rapid intravenous injections of large volumes of fluid in normal subjects, were at most only slight, in no way alters the clinical concept that when it is necessary to administer fluids intravenously in patients with a

TABLE II

Effect of intravenous infusions on ratio of functional residual and residual air volumes to total lung volume

Case	Functional residual air Total lung volume $\times 100$		Residual air Total lung volume $\times 100$	
	Before	After	Before	After
1	48.3	48.3	33.6	35.7
2	44.0	46.4	28.8	32.3
3	50.4	49.7	35.4	34.6
4	42.6	42.0	26.0	29.2
5	46.5	51.6	32.5	38.1
6	48.4	49.1	21.4	22.3

tendency toward pulmonary congestion and edema, because of cardiac, pulmonary, central nervous system, or renal disease, these infusions should be given at slower rates and with caution.

BIBLIOGRAPHY

1. Gilligan, D. R., Altschule, M. D., and Volk, M. C., The effects on the cardiovascular system of fluids administered intravenously in man. I. Studies of the amount and duration of changes in blood volume. *J. Clin. Invest.*, 1938, 17, 7.
2. Altschule, M. D., and Gilligan, D. R., The effects on the cardiovascular system of fluids administered intravenously in man. II. The dynamics of the circulation. *J. Clin. Invest.*, 1938, 17, 401.
3. Gilligan, D. R., Altschule, M. D., and Linenthal, A. J., Effects on the cardiovascular system of fluids administered intravenously in man. III. Studies of the glomerular filtration rate as measured by the urea clearance. *Arch. Int. Med.*, 1939, 64, 505.
4. Christie, R. V., The lung volume and its subdivisions. I. Methods of measurement. *J. Clin. Invest.*, 1932, 11, 1099.
5. Iglauer, A., and Altschule, M. D., The effect of arterial and venous constriction induced by paredrine (*p*-hydroxy-*a*-methylphenylethylamine hydrobromide) on the lung capacity and its subdivisions. *Am. J. M. Sc.*, 1941, 201, 664.
6. Richards, D. W., Jr., Caughey, J. L., Cournand, A., and Chamberlain, F. L., Intravenous saline infusion as a clinical test for right-heart and left-heart failure. *Tr. A. Am. Physicians*, 1937, 52, 250.
7. Christie, R. V., and Meakins, J. C., Intrapleural pressure in congestive heart failure and its clinical significance. *J. Clin. Invest.*, 1934, 13, 323.
8. Warthen, H. J., Massive intravenous injections. An experimental study. *Arch. Surg.*, 1935, 30, 199.
9. Cohnheim, J., and Lichtheim, L., Ueber Hydrämie und hydrämisches Oedem. *Virchows Arch. f. path. Anat. u. Physiol.*, 1877, 69, 106.
10. Hirshfeld, S., Hyman, H. T., and Wanger, J. J., Influence of velocity on the response to intravenous injections. *Arch. Int. Med.*, 1931, 47, 259.
11. Cournand, A., and Richards, D. W., Jr., Pulmonary insufficiency. II. The effects of various types of collapse therapy upon cardiopulmonary function. *Am. Rev. Tuberc.*, 1941, 44, 123.
12. Kraus, F., Ueber Lungenödem. *Ztsch. f. exp. Path. u. Therap.*, 1913, 14, 402.
13. Brunn, F., Experimentelles zum Lungenödem. *Wien. klin. Wchnschr.*, 1933 46, 262.
14. Farber, S., Neuropathic pulmonary edema. Further observations. *Arch. Path.*, 1940, 30, 180.

REDUCTION OF BLOOD PRESSURE ASSOCIATED WITH THE PYROGENIC REACTION IN HYPERTENSIVE SUBJECTS¹

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The use of inulin for the measurement of glomerular filtration in man was introduced in 1934 (1). It was subsequently observed that some samples of inulin may be heavily contaminated with a pyrogenic substance (2, 3), probably of bacterial origin, which induces, in addition to the commonly observed chill and fever, a marked renal hyperemia (4, 5, 6) and, particularly in hypertensive subjects, a fall in blood pressure. A suitable course of amidopyrine, administered prior to the injection of pyrogenic inulin, prevents the occurrence of the chill and fever without preventing the renal hyperemia (5, 7) and fall in blood pressure. It has been our experience that renal hyperemia and reduction of blood pressure also follow the intravenous administration of other pyrogenic substances, such as glucose, distilled water (with salt), and commercial saline, as well as triple typhoid vaccine, and it seems probable that the physiological response is generic.

In the theory that renal ischemia is the primary causal factor in the genesis of hypertension, it would be supposed that the repeated induction of renal hyperemia might have a favorable effect upon the hypertensive process. With the intent of examining this point, we repeatedly administered pyrogenic inulin to several hypertensive patients for a period of some days or weeks; a sustained reduction in blood pressure was in fact obtained, but the type of blood pressure response, coupled with other information reported below, has led us to conclude that the hypotensive action of pyrogenic inulin, as of the other pyrogens discussed in this paper, does not represent a fundamental correction of the hypertensive process but rather a complex response on the part of the circulatory system. Since pyrogens are a frequent contaminant of materials of organic origin intended for parenteral administration, we feel that the observations reported below may have some

practical value, in addition to their inherent physiological interest.

Some of the observations recorded here are drawn from our general experience, but the paper is primarily concerned with special observations made on nine subjects, eight with essential hypertension and one with chronic diffuse glomerulonephritis. These subjects were selected from the Nephritis and Hypertension Clinic of the New York University College Clinic and the Third (New York University) Medical Division of Bellevue Hospital. While in the hospital they were confined to bed rest for a period of ten to twenty days, until the blood pressure, which was determined by the auscultatory method at frequent intervals throughout the day, had become stabilized. Three subjects (M. G., F. T., R. L. in 1939) received pyrogenic inulin intravenously, and four subjects (F. K., 1939, P. K., M. T. and S. D., 1941) received triple typhoid vaccine intravenously. While this study was in progress, the effect of subcutaneous injections of tyrosinase (8, 9, 10) on renal blood flow and blood pressure was being studied in three hypertensive subjects (A. B., M. T. and F. T.), and since all three exhibited a febrile reaction during treatment, they are included in this report. We are also including observations on the blood pressure in one subject (K. S.) who developed a febrile reaction following cystoscopic examination.

PYROGENIC INULIN

What we consider to be a fairly typical picture of the immediate blood pressure response to a single dose of pyrogen (Pfanstiehl inulin, lot no. 268) in a hypertensive subject is illustrated by R. L., as shown in Figure 1. This patient was one who received repeated treatment with pyrogenic inulin, but only the first two occasions are illustrated. On the first occasion, as shown in Figure 1, the renal blood flow was followed by

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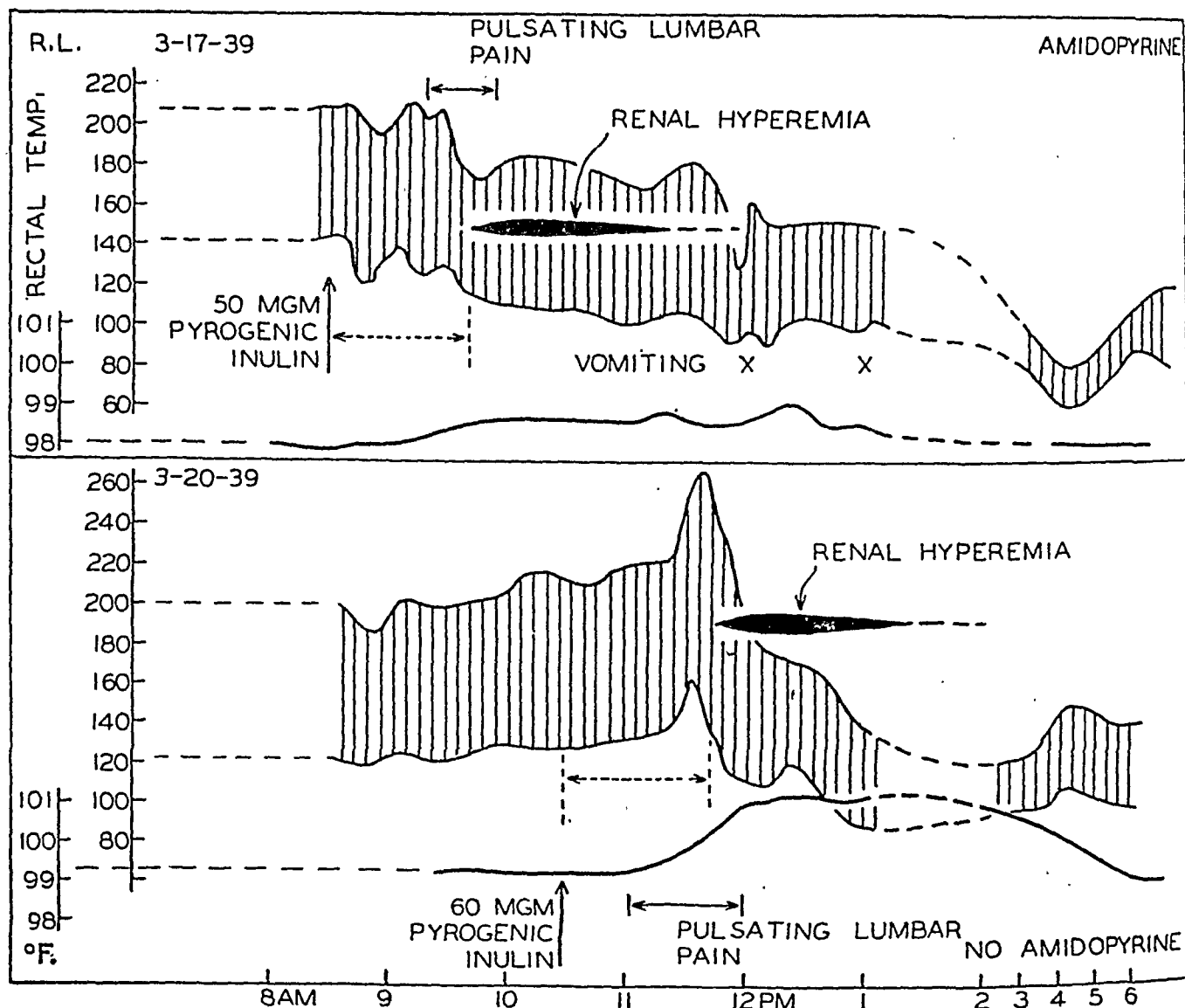


FIG. 1. HYPOTENSIVE EFFECT OF PYROGENIC INULIN ADMINISTERED INTRAVENOUSLY TO A HYPERTENSIVE SUBJECT, WITH AND WITHOUT PREMEDICATION WITH AMIDOPYRINE

The renal plasma flow was followed on the first occasion only, but it is fairly reproducible in respect to time and consequently has been interpolated in the lower graph. Note the very low pressure period which follows long after renal hyperemia is over. This subject never developed a marked fever under continued treatment.

means of the clearance method; as reported elsewhere (4, 5), a latent period of sixty to ninety minutes supervenes before the development of renal hyperemia. Renal clearances were not followed on the second occasion, but we have indicated the probable time of appearance and duration, as judged from our experience with other patients.

The blood pressure response in patients not premedicated with amidopyrine usually shows three more or less distinct phases: (a) a fleeting pressor phase, which appears from sixty to ninety minutes after pyrogen injection (see Figure 1 bottom, and also Figure 6 of Chasis *et al.* (4)

and Figure 4 of Smith (5)); (b) a phase characterized by a moderate fall in diastolic and systolic pressures, but with a well maintained pulse pressure, which immediately follows (a) and coincides roughly with renal hyperemia; and (c) a low pressure phase characterized by marked reduction in both systolic and diastolic pressures and a shallow pulse pressure, which appears four to six hours after the injection of pyrogen and which may last for several hours. The degree of blood pressure reduction in this delayed phase is highly variable, the phenomenon being slight or absent in normal subjects.

As shown in Figure 1, premedication with

amidopyrine (0.6 grams every four hours for five doses) blocks the pressor phase (a) and the rise in body temperature, but it does not block the renal hyperemia and the simultaneous changes in blood pressure described under (b), nor the delayed extreme reduction in blood pressure (c). With or without amidopyrine, the delayed reduction in blood pressure (c) may be so prolonged that the pressure is still at reduced levels the next morning.

By the repeated administration of moderate amounts of pyrogen daily, the blood pressure in hypertensive subjects can be maintained at reduced levels. Figure 2A (M. G.) shows the effects of daily administration of pyrogenic inulin. This patient received treatment during two periods of twelve and eleven days respectively, each injection (50 mgm. to 300 mgm. inulin lot no. 268) being followed by a febrile reaction. During the administration of pyrogen, the daily average blood pressure (six or more readings exclusive of the pressor phase) remained at significantly lower levels than during the control period, and the hypotensive effect persisted for the twelve days of observation following the last injection. To what extent the maintained reduction in blood pressure is attributable to the persistence of the more acute hypotensive action described under (c), or to other and unknown factors, cannot be said.

In two other patients, a similar sustained reduction in blood pressure was produced by the repeated intravenous administration of pyrogenic inulin. In none of these was there evidence of immediate or delayed (two years) injurious effect, as discoverable by repeated urinalyses and renal clearance tests.

TRIPLE TYPHOID VACCINE

Figure 2B (F. K.) shows the effect on the blood pressure of repeated administration of triple typhoid vaccine intravenously. Sixteen injections (0.05 to 5.5 cc. of New York City Department of Health standard vaccine) were given over a period of thirty-eight days, each injection being followed by a febrile reaction. The blood pressure fell markedly following the first injection and was maintained at a low level throughout the period of treatment.

Two other hypertensive subjects were given

triple typhoid vaccine intravenously. P. K. was treated daily for eleven days with a sustained hypotensive effect. The results are similar to those obtained on F. K. and need not be illustrated.

Patient S. D. merits detailed discussion. She was a sixty-year-old white female whose hypertension had been discovered at the age of fifty-two. There was no history of diminution in cardiac reserve and no clinical evidence of congestive heart failure. Examination of the ocular fundi showed in moderate degree the vascular changes associated with hypertension. The blood pressure ranged from 244/136 to 164/110 mm. Hg during a ten-day control period on bed rest. Proteinuria and hematuria were absent; the specific gravity of the urine ranged from 1025 to 1005; the urea clearance was within the normal range and the blood non-protein nitrogen was 35 mgm. per cent. The administration of amidopyrine (0.6 grams every four hours for five doses) on the first day of premedication produced nausea and vomiting; triple typhoid vaccine (0.02 cc.) was nevertheless given intravenously on the second day and induced a reaction consisting of severe bilateral lumbar pain and pain in left anterior chest. The rectal temperature remained at 98.8° F. and the blood pressure level did not change. Amidopyrine was continued during the third day, but no vaccine was given. Amidopyrine was continued for the fourth day and triple typhoid vaccine again was withheld; the nausea and vomiting had now subsided and the patient was comfortable. On the fifth day, still continuing amidopyrine, the intravenous administration of vaccine (0.05 cc.) was followed in one hour by violent throbbing lumbar pain and vomiting, and three hours after the injection the patient went into peripheral circulatory failure and became unconscious. The blood pressure fell to 80/52 mm. Hg, the heart rate remaining at 78 to 86 throughout the reaction. The patient recovered consciousness in half an hour, the blood pressure rising to 130/76, only to fall again in seven hours from the injection to 70/50, with relapse into unconsciousness, accompanied by pallor and sweating. Consciousness was shortly recovered and in an hour the circulatory status had improved. The patient fully recovered from the episode, and in

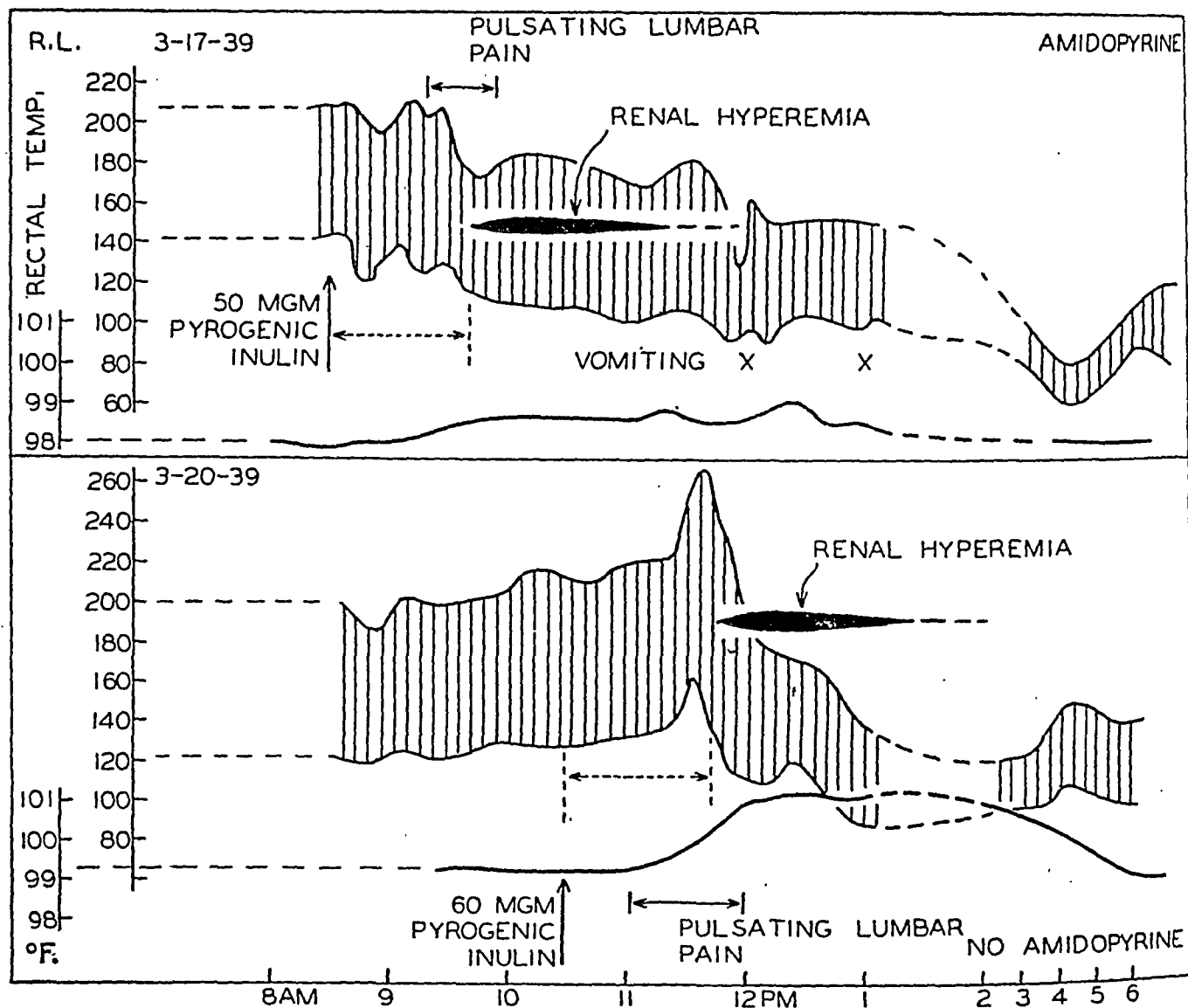


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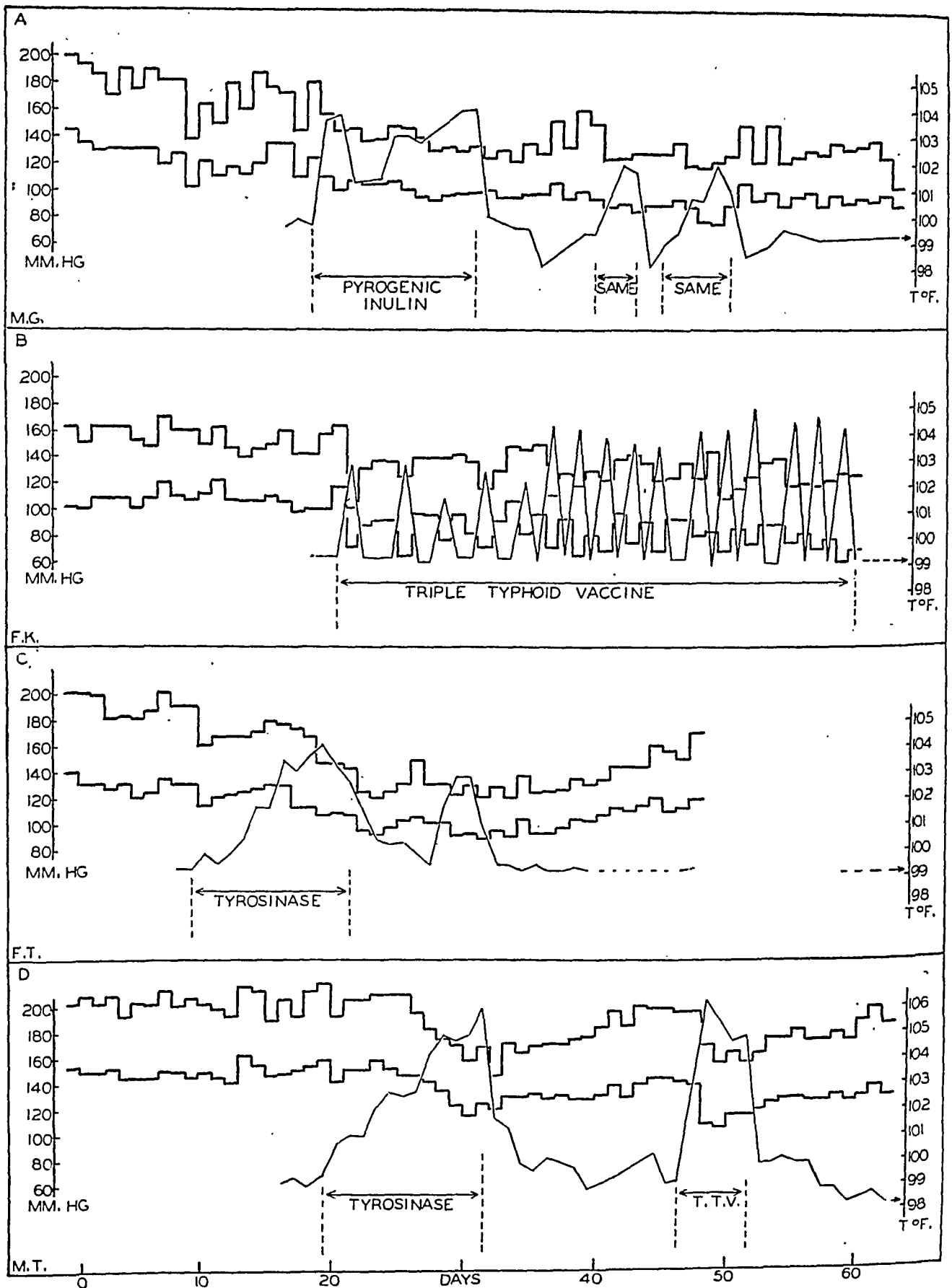


FIG. 2. BLOOD PRESSURE AND RECTAL TEMPERATURE IN SUBJECTS RECEIVING PYROGENIC AGENTS

A. Prolonged hypotensive effect of repeated intravenous injections of pyrogenic inulin in a subject with essential hypertension.

three days the blood pressure had returned to the control level.

Since amidopyrine was continued throughout treatment without ill-effects certainly attributable to this drug, and with recovery from nausea, and since the episode of circulatory failure followed the administration of triple typhoid vaccine, we believe that this patient illustrates an instance where the hypotensive action of pyrogenic material itself resulted in dangerous embarrassment of the circulatory system. It may be specially noted that there was no fever at any time during the five days of treatment.

TYROSINASE

Tyrosinase was administered subcutaneously in the thigh to two subjects with essential hypertension (A. B., not illustrated, and M. T.), and to one subject (F. T.) in the hypertensive stage of chronic diffuse glomerulonephritis. In all three there developed, at the site of injection, local reactions varying from a localized area of pigmented erythema to a widespread, edematous, tender, cellulitis-like area with inguinal adenopathy. In all three subjects during the period of treatment there occurred persistent fever, associated with malaise, prostration, anorexia, dehydration, and loss of weight.

Figure 2C records the effect of tyrosinase (1300 to 7800 catacolase units)² on the blood pressure of subject F. T. The injections were given daily for twelve days. With the development of fever, the blood pressure fell to significantly lower levels and tended to return to the control level as the local reactions at the site of injection subsided and the body temperature returned to normal. (The second rise in temperature, after the injections had been discontinued, was associated with continuing severe local reaction.)

² Two weeks after the end of this therapy, the tyrosinase preparation had lost 40 per cent of its catacolase activity. The pH (7.3) had not changed.

We are indebted to Dr. John M. Nelson of Columbia University for supplying the tyrosinase.

Figure 2D shows the response of the subject (M. T.) with essential hypertension, who was treated on two occasions: first, with tyrosinase (see F. T. for dosage) administered subcutaneously; and after a second control period in which the blood pressure had returned to its original hypertensive level, with triple typhoid vaccine (1.0 to 3.0 cc.), administered intravenously. A hypotensive effect was produced on both occasions.

In all three patients treated with tyrosinase, the blood pressure fell only after elevation of body temperature had occurred.

POST-CYSTOSCOPIC REACTION

Figure 3 (K. S.) shows the blood pressure response in a patient with essential hypertension during a post-cystoscopic febrile reaction. Five hours following cystoscopy the patient had a severe chill lasting for one-half hour, followed by a rise in body temperature which then remained constantly elevated. The blood pressure started to fall following the first chill and remained at a reduced level throughout the febrile period. The

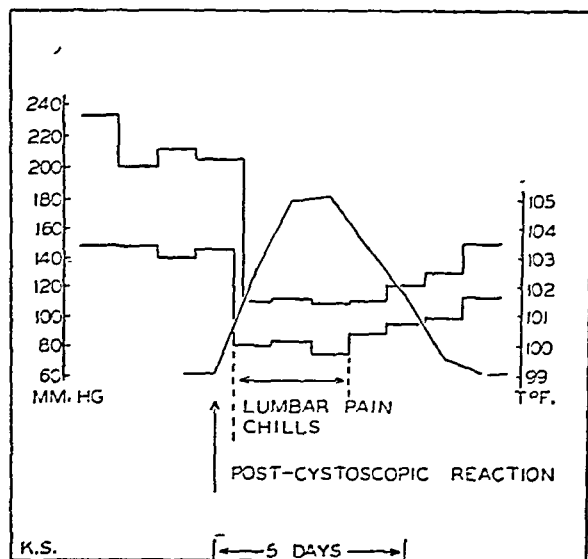


FIG. 3. MARKED FALL IN BLOOD PRESSURE ASSOCIATED WITH A POST-CYSTOSCOPIC FEBRILE REACTION IN A SUBJECT WITH ESSENTIAL HYPERTENSION

B. Prolonged hypotensive effect of repeated intravenous injections of triple typhoid vaccine in a subject with essential hypertension.

C. Prolonged hypotensive effect of repeated subcutaneous injections of tyrosinase in a subject in the hypertensive stage of chronic diffuse glomerulonephritis.

D. Prolonged hypotensive effect of repeated subcutaneous injections of tyrosinase, followed by a second period of treatment with triple typhoid vaccine, in a subject with essential hypertension.

patient did not show signs of circulatory inadequacy during the hypotensive phase and had no subjective complaints after the fever had subsided.

It is not uncommon for acute infectious febrile states to be accompanied by a fall in blood pressure in hypertensive subjects, a phenomenon which is generally recognized. The subject K. S. is reported here chiefly as a striking instance of this response.

DISCUSSION

The pyrogenic reaction, although it can be induced in all subjects, varies not only in its manifestations but also in its severity in different subjects. Characteristically, it consists of a sensation of chilliness or a severe chill, pulsating lumbar pain, headache, fever, and renal hyperemia, with the sequence of complex vasomotor responses briefly described earlier in this paper. In some individuals there may also occur nausea, vomiting, substernal pain, cyanosis, and, in occasional instances, peripheral circulatory failure. Premedication with amidopyrine blocks the chill, the pressor phase in the vasomotor response, the rise in body temperature, and all subjective symptoms except occasionally the pulsating lumbar pain, without blocking the renal hyperemia or the fall in blood pressure which, as represented by the second phase, coincides with the renal hyperemia, or as represented by the third phase, consists of a more severe hypotensive action, long outlasting the renal hyperemia. During this prolonged hypotensive action, the blood pressure may fall to alarmingly low levels; this phenomenon is, however, variable in its occurrence and severity, and cannot be predicted.

Pyrogen itself is apparently not a depressor substance or vasodilator, since it has no perceptive immediate action on blood pressure, as determined by the auscultatory method, or on renal blood flow; and a latent period of about ninety minutes typically intervenes before the blood pressure begins to fall. The vasomotor responses occurring in patients receiving pyrogen are undoubtedly complex, and also probably differ in normal and in hypertensive subjects.³ The initial pressor

phase appears to be a neurogenic vasoconstriction associated with the autonomic disturbance which is elicited by pyrogenic agents in general. The second phase, wherein both systolic and diastolic pressures are moderately reduced, with typically a wide pulse pressure, perhaps reflects the decrease in peripheral resistance associated with the observed dilatation of the renal vascular bed. Dilatation in other organs cannot, of course, be excluded. The more severe reduction in blood pressure which occurs after several hours, and which, as we have said, is variable and unpredictable, and more severe in hypertensive than in normal subjects, may represent a more extreme state of vasodilatation but the reduction in pulse pressure suggests the presence of a diminished cardiac output or other adverse reactions in the circulatory system.

The pyrogenic reaction has been observed by us to follow the administration of triple typhoid vaccine, pyrogenic inulin, and tyrosinase, and it has also been observed during a post-cystoscopic febrile reaction. We have also seen the reduction in blood pressure following the intravenous administration of pyrogenic normal saline and glucose. Though different pyrogens may be involved in these reactions, it is well known that a pyrogenic substance is characteristic of Gram-negative bacteria, and it has been demonstrated that a substance can be extracted from *D. dysenteriae* and *B. coli*, which is composed of a polysaccharide, a phospholipin, and a polypeptide-like molecule (11, 12), and which is capable of producing a reaction in rabbits similar in its general effects to the one described in this study.⁴ Since many post-cystoscopic reactions are the result of a transient *B. coli* bacteremia,⁵ it may be that substances arising in Gram-negative bacteria are the common active factors in all the pyrogenic reactions discussed above.

⁴ Personal communication from Dr. Rene J. Dubos. The examination of the physiological effect of these fractions is now in progress, in collaboration with Dr. Dubos.

⁵ A bacillus of the *B. coli* group was found to be the invasive organism in 17 of 25 patients who had a bacteremia following cystoscopy, in work to be reported by Dr. Justina Hill of the James Buchanan Brady Urological Institute, Johns Hopkins Hospital. This is not meant to indicate that every post-cystoscopic febrile reaction is associated with a bacteremia.

³ These hemodynamic responses are being studied in more detail at the present time, particularly with reference to cardiac output.

Our observations on tyrosinase are included in this report because of the relationship between reduction of blood pressure and fever; in no case did we observe blood pressure to be reduced until the local reaction had gone far enough to induce some febrile response. Our results, however, do not demonstrate that tyrosinase is without specific effect on blood pressure, and the fact that heating destroys the hypotensive action of tyrosinase preparations (Schroeder, personal communication), where pyrogen is generally resistant to heating, argues in favor of a specific action.

Bacterial contamination is allegedly responsible for the pyrogenic properties of some samples of distilled water; and any material prepared chemically with ordinary water, which itself is frequently contaminated with pyrogenic organisms, must be suspected. It has been our experience that once inulin is contaminated with pyrogen, repeated purification by recrystallization from water or alcohol-water mixtures fails to remove the pyrogen, which can be removed only by effective absorbants; and if organic preparations intended for parenteral administration were once contaminated with pyrogen, either from the raw materials, the water used in manufacture, or by bacterial growth during the course of preparation, effective quantities might be carried through repeated purifications. It is also possible that the local tissue reaction which sometimes follows the injection of foreign material may give rise to an endogenous factor having a vasomotor action similar to that of bacterial pyrogen, since such local reactions are frequently accompanied by fever.

The fact that amidopyrine blocks the rise in body temperature, without blocking the hypotensive action and the renal vasodilatation induced by pyrogenic agents, may bespeak a dual nature in pyrogen itself, or merely a dissociation of the febrile and vascular responses within the body. This, like many other problems associated with the pyrogenic reaction, invites further investigation, but, until the question is answered, it seems unwarranted to accept the febrile response itself as an adequate criterion of the presence or absence of powerful, delayed-action, vasomotor agents of the type studied here.

We have observed no clinical signs of injurious action in the patients treated here, as judged by

urinalyses and renal clearance studies, but it is known that bacterial extracts administered in closely repeated doses may produce delayed necrotic lesions in the kidneys (Shwartzman phenomenon (13)), and such substances should be administered with due consideration of this fact. It should also be noted that pyrogenic material may induce an alarming circulatory crisis, as illustrated by our patient S. D., in consequence of the delayed-action vasomotor effects.

SUMMARY

Blood pressure can be reduced significantly in hypertensive subjects by the intravenous administration of pyrogenic material (pyrogenic inulin, triple typhoid vaccine, tyrosinase), and it can be maintained at reduced levels by the repeated injections of this material. This hypotensive effect can be obtained without a rise in body temperature by premedication with amidopyrine.

The mechanism responsible for the persistent blood pressure reduction is unknown, but, from the more immediate effects of pyrogen, it appears to be attributable in part to an adverse or asthenic action on the cardiovascular system, rather than a correction of the fundamental disturbance underlying the hypertensive process.

One instance of a marked reduction in blood pressure in a hypertensive subject during a post-cystoscopic febrile reaction is illustrated. Such reactions are reported to be attributable to a transient *B. coli* bacteremia, and the reduction of blood pressure here, and in other acute infections, may be associated with the pyrogenic reaction associated with the infection.

Whenever the blood pressure of a hypertensive subject is reduced by the parenteral administration of a foreign organic material, this pyrogenic type of response should be excluded before a specific hypotensive property is attributed to the agent used. And any pyrogenic material should be administered cautiously, since it may induce an alarming degree of peripheral circulatory failure, as illustrated by one of our subjects (S. D.).

BIBLIOGRAPHY

1. Shannon, J. A., and Smith, H. W., The excretion of inulin, xylose and urea by normal and phlorizinized man. *J. Clin. Invest.*, 1935, 14, 393.

2. Goldring, W., and Smith, H. W., Inulin and its suitability for intravenous administration in man. *Proc. Soc. Exper. Biol. and Med.*, 1936, 34, 67.
3. Smith, H. W., Chasis, H., and Ranges, H. A., Suitability of inulin for intravenous administration to man. *Proc. Soc. Exper. Biol. and Med.*, 1938, 37, 726.
4. Chasis, H., Ranges, H. A., Goldring, W., and Smith, H. W., The control of renal blood flow and glomerular filtration in normal man. *J. Clin. Invest.*, 1938, 17, 683.
5. Smith, H. W., The physiology of the renal circulation. *Harvey Lect.*, 1939-40, 35, 116.
6. Smith, H. W., Chasis, H., Goldring, W., and Ranges, H. A., Glomerular dynamics in the normal human kidney. *J. Clin. Invest.*, 1940, 19, 751.
7. Goldring, W., Chasis, H., Ranges, H. A., and Smith, H. W., Effective renal blood flow in subjects with essential hypertension. *J. Clin. Invest.*, 1941, 20, 637.
8. Schroeder, H. A., Effect of tyrosinase on blood pressure of hypertensive rats. *Proc. Soc. Exper. Biol. and Med.*, 1940, 44, 172.
9. Schroeder, H. A., The effect of tyrosinase on arterial hypertension. *Science*, 1941, 93, 116.
10. Schroeder, H. A., Adams, M. H., and Cohn, A. E., The effects of tyrosinase on arterial hypertension. *J. Clin. Invest.*, 1941, 20, 442.
11. Boivin, A., Mesrobian, L., Magheru, G., and Magheru, A., Recherches biologiques et chimiques sur l'antigène somatique "complet" renfermé dans quelques colibacilles. *Compt. rend. Soc. de biol.*, 1935, 120, 1276.
12. Morgan, W. T. J., and Partridge, S. M., Studies in immunochemistry. 4. Fractionation and nature of antigenic material isolated from *Bact. dysenteriae* (Shiga). *Biochem. J.*, 1940, 34, 169.
13. Apitz, K., A study of the generalized Shwartzman phenomenon. *J. Immunol.*, 1935, 29, 255.

THE RELATION BETWEEN DARK ADAPTATION AND THE LEVEL OF VITAMIN A IN THE BLOOD

BY CHARLES HAIG AND ARTHUR J. PATEK, JR.

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The purpose of the present study is to determine if a relation exists between dark adaptation measurements and the level of vitamin A and total carotenoids in the blood plasma, in normal subjects and in patients with cirrhosis of the liver.

It has been established that the retina's ability to adapt to the dark depends upon an adequate supply of vitamin A from the diet. The evidence has been reviewed by Wald and his co-workers (1), and by Hecht and Mandelbaum (2). Although failures to observe any influence of vitamin A intake upon dark adaptation have been recorded (3 to 5), other studies of experimental vitamin A deficiency (1, 2, 6 to 10) have shown that the visual threshold at complete dark adaptation can be elevated greatly by reduced vitamin A intake. In the latter studies the rate of dark adaptation remained unaltered.

It has been shown previously (11 to 15) that patients with cirrhosis of the liver may have greatly delayed dark adaptation, with or without elevation of the final threshold. Since in these patients the response to vitamin A therapy was characterized by an increase in the rate of dark adaptation and a decrease in the final threshold (when originally high), it was concluded that there exists in cirrhosis of the liver a disturbance of vitamin A metabolism which differs from ordinary vitamin A deficiency.

It also has been shown that low values for vitamin A are found in the plasma and liver of experimental animals with vitamin A deficiency (16, 17). Moreover, the livers of rats with carbon tetrachloride cirrhosis have been shown to contain only half the amount of vitamin A present in the livers of normal animals fed the same quantity of food and of vitamin A (18). Likewise, in patients with cirrhosis of the liver, low values for vitamin A are found in both the circulating plasma (19 to 22) and in the liver tissue at autopsy (22 to 26). Since both dark adaptation and the

level of plasma vitamin A are related directly to the state of nutrition with reference to vitamin A, it seemed reasonable to expect a degree of correlation between the two types of measurement.

METHODS

The apparatus and the technique here employed for measuring the dark adaptation function have been described elsewhere (12, 27, 28). The intensity of the white pre-adapting light was 6,000 millilamberts, and was viewed by the subject with the right eye for 3 minutes. The test light, a flash of 0.2 second duration, passed through a violet filter (Corning No. 511). The retinal region tested was a circular area whose diameter subtended a 2° visual angle and was located 5° nasally to the fovea of the right eye of the subject. Both the pre-adapting light and the test flash were viewed through a 2 mm. artificial pupil placed at a distance of 3 mm. before the cornea of the subject.

The plasma level of vitamin A and total carotenoids were determined by a modification of the method described by Kimble (29). It was found that shaking the plasma sample for 15 minutes with the ethanol before adding petroleum ether insured more complete precipitation of the proteins and more thorough extraction of the vitamin A and carotenoids. It was also discovered that using the chloroform and the antimony trichloride solution at a low temperature (*circa* 10° C.) delays the development and fading of the blue color of the vitamin A-SbCl₃ reaction sufficiently to permit several readings before the maximum density is attained and passed, thus making possible a more exact estimate of the maximum value. The densities were measured in a Bausch and Lomb spectrophotometer. The vitamin A and carotenoid levels were expressed as international units (I.U.) and micrograms (μgm.), respectively, per 100 ml. of plasma. Whenever a sufficient quantity of plasma was available, duplicate determinations were made.

All of the patients received highly nutritious diets which were estimated from food tables (30) to provide at least 13,000 I.U. of vitamin A daily. None had fever, jaundice, or diarrhea at the time the tests were made. The observed abnormal values, therefore, are not attributable to low intake of the vitamin, to fever, nor to faulty gastrointestinal absorption due to jaundice or diarrhea.

RESULTS

In Figure 1 are plotted the upper and lower limits of the data of 60 individual dark adaptation tests made on 37 normal persons between the ages of 20 and 45 years. The abscissae are minutes in the dark after cessation of light adaptation, and the ordinates are the logarithms of the threshold intensities expressed in micromicrolamberts ($\mu\mu\text{L}$). The *final threshold* is the lowest threshold reading obtained during a stay in the dark sufficiently long to define the entire rod function. The *adaptation time* is defined as the number of minutes in the dark required for the dark adaptation function to attain a threshold level of 5.50. This parameter obviously possesses both velocity and threshold-level dimensions, and thus serves as an over-all index of the subject's dark adaptation status.

Values for the adaptation time and for the final threshold of the 37 normal persons are given in Table I. When more than one observation was made on an individual, the mean value is given, and the number of observations indicated in parentheses. The adaptation time ranges in value from an average of 9.5 minutes to 15.0

minutes, with a mean of 13.1 minutes. The final threshold values range from an average of 3.95 to 4.42, with a mean of 4.20. For unexplained reasons the values for a single individual may vary in exceptional cases by as much as 3 minutes and 0.4 log unit over a period of several months. However, the usual limits of change over a period of 2 or 3 weeks are approximately ± 0.5 minute and ± 0.2 log unit.

Within the age limits studied, neither sex nor age appear to exert a significant influence upon either the adaptation time or the final threshold.

The plasma vitamin A values found in Table I are based upon 74 measurements on 44 normal persons between the ages of 20 and 45 years. The values range from 109 to 309 I.U. per cent and have a mean value of 198 I.U. per cent. The amount of variation in single individuals over a period of months may be almost as great as the individual differences shown in the table. Changes as great as 50 per cent have been observed within a period of only one week. This instability of the vitamin A blood level presents a striking contrast to the relative constancy of the dark adaptation function. It is accounted for in part

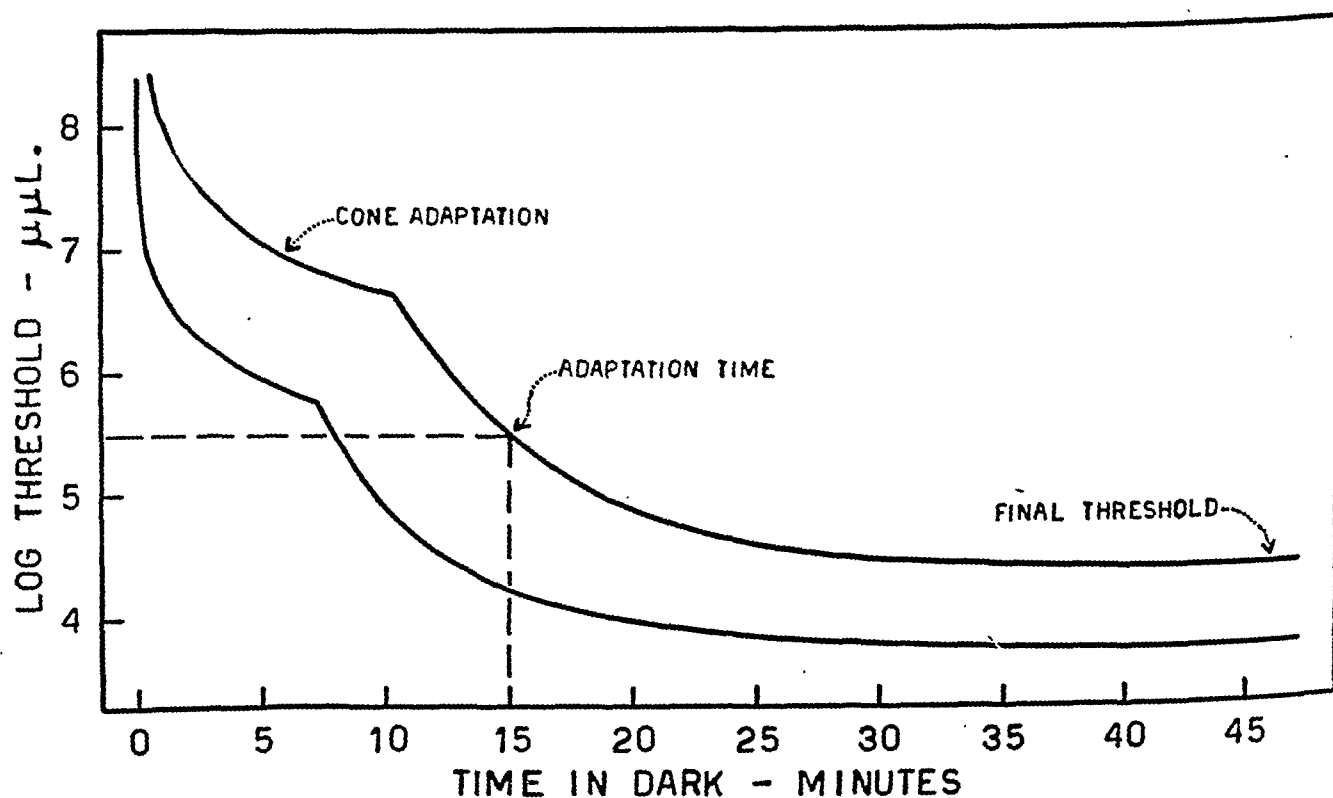


FIG. 1. THE LIMITS OF 60 DARK ADAPTATION MEASUREMENTS IN 37 NORMAL SUBJECTS

Average values of the parameters designated *adaptation time* and *final threshold* for each subject are recorded in Table I.

TABLE I

Plasma vitamin A, plasma carotenoids, dark adaptation time, and final threshold intensity in normal persons
(When the value represents an average, the number of observations is indicated in parentheses.)

Males					Females				
Subject	Plasma vitamin A	Plasma carotenoids	Adaptation time	Final threshold	Subject	Plasma vitamin A	Plasma carotenoids	Adaptation time	Final threshold
	I.U. per 100 ml.	μgm. per 100 ml.	minutes	log μl.		I.U. per 100 ml.	μgm. per 100 ml.	minutes	log μl.
1M	189 (7)	140 (7)	9.5 (3)	4.18 (3)	1F	149 (2)	106 (2)	12.0 (3)	4.03 (3)
2M	197 (4)	150 (4)	11.3 (3)	4.02 (3)	2F	176 (2)	156 (2)	13.0 (2)	3.97 (2)
3M	205 (7)	185 (7)	15.0 (2)	4.29 (2)	3F	213	104	14.9 (2)	4.15 (2)
4M	180 (2)	106 (2)	14.2	4.30	4F	135	113	11.8	4.10
5M	193 (2)	117 (2)	12.4 (2)	4.25 (2)	5F	172 (3)	110 (3)	10.9 (4)	4.10 (4)
6M	159	174	10.7	4.00	6F	273	174		
7M	260 (2)	69 (2)			7F	186 (2)	135 (2)	15.0	4.40
8M	309	146 (2)	14.0	4.40	8F	144	129		
9M	109	122	13.0	4.00	9F	198	133		
10M	229	91			10F	176	158		
11M	149 (2)	143 (2)	12.2 (2)	4.13 (2)	11F	195	154	14.2	4.30
12M	209 (2)	94 (2)			12F	158 (3)	245 (3)	13.6	4.10
13M	167	234	12.5	4.30	13F	213	125		
14M	285	149	13.8	4.00	14F	265	108		
15M	230	108	12.3 (2)	4.39 (2)	15F	125	166		
16M	280	216	14.9	4.42	16F	125	133		
17M	214	158			17F	141	154		
18M	290 (2)	260 (2)			18F	282	239		
19M	267	112	12.8	4.20	19F	114	75	13.1 (3)	4.23 (3)
20M	230	203			20F	192	108	14.2 (3)	4.30 (3)
21M	192	183			21F			12.4 (2)	3.95 (2)
22M	169	183	10.8	4.25	22F			13.2	4.20
23M			14.3	4.10	23F			14.8 (2)	4.10 (2)
24M			12.9 (2)	4.20 (2)					
25M			15.0	4.42					
26M			13.6	4.42					
27M			12.0 (2)	4.08 (2)					
28M			14.3	4.20					
29M			13.8	4.25					
30M	180	83	9.7	4.20					
31M	200	64	13.5	4.20					
Mean	212	146	12.9	4.22	Mean	182	142	13.4	4.15
Standard deviation	49.8	46.9	1.65	0.132	Standard deviation	28.3	27.4	1.23	0.198
Mean of M + F	198	144	13.1	4.20					
Standard deviation	41.5	34.8	1.57	0.142					

by a seasonal variation, possibly correlated with diet (31). However, so many departures from the seasonal trend have been noted, that, almost certainly, one or more additional unknown factors must exert an influence upon the level of vitamin A in the blood.

The difference of 30 I.U. per cent between the mean values for men and for women in the plasma vitamin A data of Table I is found to have statistical significance. This confirms similar findings by Kimble (29) and by Murrill and his co-workers (32). On the other hand, the mean plasma carotenoid levels of the two sexes are practically identical, which contrasts with the

findings of Kimble and of Murrill *et al.* of slightly higher values for women than for men.

Within the age limits studied, no significant influence of age upon the plasma vitamin A and carotenoid levels was observed.¹

¹ In another study (33), by a procedure which was calibrated against the present technique, the mean level of vitamin A in the blood of infants between 3 weeks and 6 months of age was found to be 74 I.U. per cent, that for infants between 6 and 18 months of age 110 I.U. per cent, and that for children from 6 to 12 years of age 117 I.U. per cent. When the mean of 198 I.U. per cent here obtained on adult subjects is added to this series, it is apparent that the level of plasma vitamin A rises significantly with increasing age up to the adult level.

For studying the relation between the two kinds of measurement, blood for vitamin A and carotenoid analysis was drawn on the same day that the dark adaptation tests were made. Table II presents 67 such simultaneous dark adaptation plasma measurements in 14 normal persons, 18 persons with cirrhosis of the liver, and 7 persons with various other chronic diseases. Figure 2

TABLE II

Simultaneous measurements of plasma vitamin A and carotenoids, and of dark adaptation

Sub- ject	Sex	Diagnosis	Plasma vita- min A	Plasma carote- noids	Adap- tation time	Final thres- hold
			<i>I.U. per 100 ml.</i>	<i>μgm. per 100 ml.</i>	<i>min- utes</i>	<i>log μμl.</i>
1M	M	Normal	256	116	8.5	4.10
1M	M	Normal	142	134	7.8	4.00
2M	M	Normal	175	170	10.8	3.95
2M	M	Normal	204	129	11.5	4.05
3M	M	Normal	218	176	15.0	4.42
13M	M	Normal	167	234	12.5	4.30
19M	M	Normal	267	112	12.8	4.20
22M	M	Normal	169	183	10.8	4.25
30M	M	Normal	180	64	9.7	4.20
31M	M	Normal	200	83	13.5	4.20
1F	F	Normal	181	149	9.9	4.05
2F	F	Normal	137	133	12.5	3.84
5F	F	Normal	141	96	10.9	4.10
5F	F	Normal	189	83	10.9	4.10
7F	F	Normal	134	111	15.0	4.40
19F	F	Normal	114	75	11.3	4.25
20F	F	Normal	192	108	13.6	4.10
3C	M	Cirrhosis of the liver	122	116	23.5	4.89
3C	M	Cirrhosis of the liver	146	125	16.5	4.25
3C	M	Cirrhosis of the liver	161	134	21.5	4.40
3C	M	Cirrhosis of the liver	192	212	21.0	4.60
3C	M	Cirrhosis of the liver	147	141	22.8	4.55
3C	M	Cirrhosis of the liver	75	174	23.8	4.50
3C	M	Cirrhosis of the liver	90	224	23.0	4.30
3C	M	Cirrhosis of the liver	106	241	22.5	4.95
3C	M	Cirrhosis of the liver	85	220	19.7	3.90
3C	M	Cirrhosis of the liver	180	133	20.7	4.30
3C	M	Cirrhosis of the liver	98	116	18.9	3.90
3C	M	Cirrhosis of the liver	193	187	18.7	3.30
6C	M	Cirrhosis of the liver	105	215	14.3	4.50
9C	M	Cirrhosis of the liver	116	145	16.2	4.70
12C	F	Cirrhosis of the liver	24	216	18.2	4.20
12C	F	Cirrhosis of the liver	74	73	25.0	4.25
15C	F	Cirrhosis of the liver	32	125	29.0	4.75

TABLE II—Continued

Sub- ject	Sex	Diagnosis	Plasma vita- min A	Plasma carote- noids	Adap- tation time	Final thres- hold
			<i>I.U. per 100 ml.</i>	<i>μgm. per 100 ml.</i>	<i>min- utes</i>	<i>log μμl.</i>
15C	F	Cirrhosis of the liver	47	133	26.5	4.20
16C	F	Cirrhosis of the liver	62	291	21.8	4.25
16C	F	Cirrhosis of the liver	100	179	19.8	4.40
16C	F	Cirrhosis of the liver	63	270	17.0	4.40
33C	F	Cirrhosis of the liver	161	66	12.5	4.00
36C	M	Cirrhosis of the liver	47	135	19.0	4.10
36C	M	Cirrhosis of the liver	49	91	17.5	4.20
36C	M	Cirrhosis of the liver	44	83	19.4	4.25
37C	F	Cirrhosis of the liver	93	166	14.5	4.80
37C	F	Cirrhosis of the liver	27	66	14.0	5.00
54C	M	Cirrhosis of the liver	57	58	18.0	4.30
54C	M	Cirrhosis of the liver	60	57	19.4	4.50
55C	M	Cirrhosis of the liver	87	44	15.1	4.10
56C	F	Cirrhosis of the liver	116	44	25.0	4.90
57C	M	Cirrhosis of the liver	164	79	10.4	4.50
58C	F	Cirrhosis of the liver	85	42	18.8	4.24
59C	F	Cirrhosis of the liver	77	36	21.0	4.40
61C	F	Cirrhosis of the liver	98	40	24.0	4.90
62C	M	Cirrhosis of the liver	89	50	12.5	4.40
63C	M	Cirrhosis of the liver	69	28	13.1	4.30
9H	F	Nephrosis	122	191	9.0	3.95
9H	F	Nephrosis	152	187	13.7	4.05
11H	F	Diabetes mellitus	199	170	16.7	4.40
27H	F	Urolithiasis	84	97	21.3	4.40
27H	F	Urolithiasis	105	56	25.0	4.35
27H	F	Urolithiasis	107	71	23.2	4.35
52H	F	Hyperthyroidism	155	75	17.7	4.25
52H	F	Hyperthyroidism	173	58	15.5	4.10
52H	F	Hyperthyroidism	133	37	13.3	3.67
52H	F	Hyperthyroidism	162	50	14.5	4.10
53H	F	Myxedema	116	145	13.0	4.16
54H	F	Myxedema	170	133	16.1	4.50
71H	M	Anomalous portal vein	166	179	14.8	4.50

shows the relation of the plasma vitamin A values to (A) the dark adaptation time, and to (B) the final threshold.

DISCUSSION

When the several groups of subjects are regarded as a *single* population, the data of Figure 2 show that the higher dark adaptation values

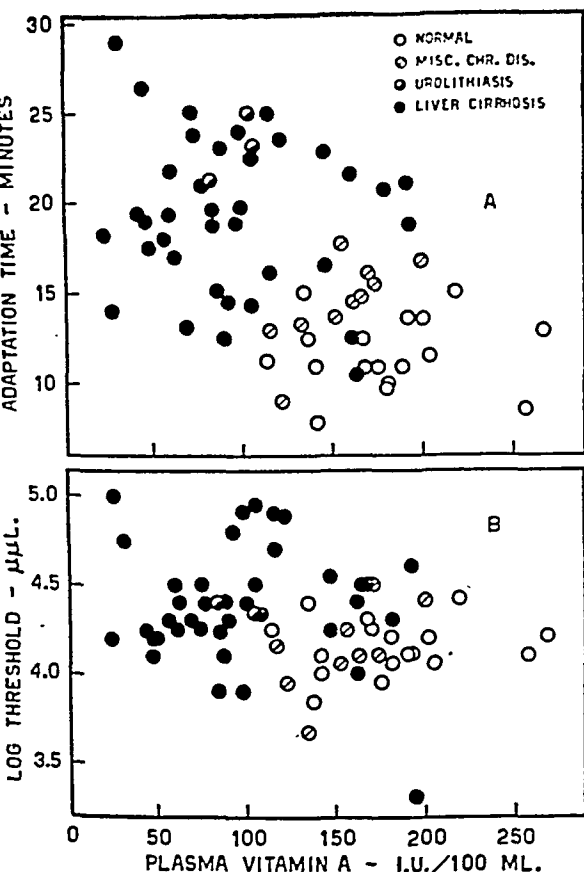


FIG. 2. RELATION OF THE PLASMA VITAMIN A LEVEL TO (A) THE DARK ADAPTATION TIME, AND TO (B) THE LOGARITHM OF THE FINAL THRESHOLD INTENSITY

The 67 points represent individual observations made on the same days in 14 normal persons, 18 patients with cirrhosis of the liver, and 7 patients with certain other diseases. Note that the data representing the several clinical categories are not randomly distributed with relation to each other, but are crudely aligned in the order: liver cirrhosis, urolithiasis, miscellaneous chronic diseases, and normal.

tend to be associated with the lower vitamin A values, suggesting a degree of correlation between the two variables. This effect is more marked in Figure 2A (adaptation time) than in Figure 2B (final threshold). When, however, the data of the cirrhotic and normal groups in this figure are regarded separately as distinct populations, they show an essentially random distribution. Within either of these clinical categories, therefore, no correlation is apparent between the plasma vitamin A level and either parameter of the dark adaptation function.

Since the data of the several groups are not randomly distributed over the same range of values, it is apparent that the correlations arise from a tendency of the data to group themselves according to the separate clinical categories, the cirrhotics being at one end of a series and the normals at the other. This tendency for patients with cirrhosis of the liver to have higher dark adaptation and lower plasma vitamin A values than normal controls has been previously observed (22). However, in the previous study the dark adaptation and vitamin A values were not measured simultaneously.

Similar correlations between dark adaptation measurements and the plasma vitamin A level have been reported previously by others. It is possible that these correlations, like those of the present data, are attributable to other factors rather than to a direct dependence of the retina upon the level of vitamin A in the blood. In the studies by Lindqvist (20), Pett and LePage (34), and Lewis *et al.* (33), heterogeneous groups of patients (miscellaneous diseases) were also employed. In that by Josephs and his co-workers (35), the subjects were drawn from four different nutritional categories as determined by questionnaires and by economic status.

Evidence that the retinal supply of vitamin A may be largely independent of the level of the vitamin in the blood is provided by the observations of Lewis and his co-workers (36), who found that the retinas of rats on a diet of low vitamin A content retained a maximal quantity of vitamin A, although the plasma level had dropped to an extremely low value. Even more striking is the finding that thyroid extract or α -dinitrophenol administered to patients with delayed dark adaptation, not only lowered the plasma vitamin A and carotenoid levels, but simultaneously *increased* the speed and extent of dark adaptation (37). Still other factors, enumerated in an earlier report (22), have been shown to influence independently either the dark adaptation or the blood vitamin A level. It nevertheless appears reasonable, when known complicating factors are excluded, to regard dark adaptation values as measures of the *utilization* of vitamin A by the retina. The level of vitamin A in the blood, on the other hand, has been shown ex-

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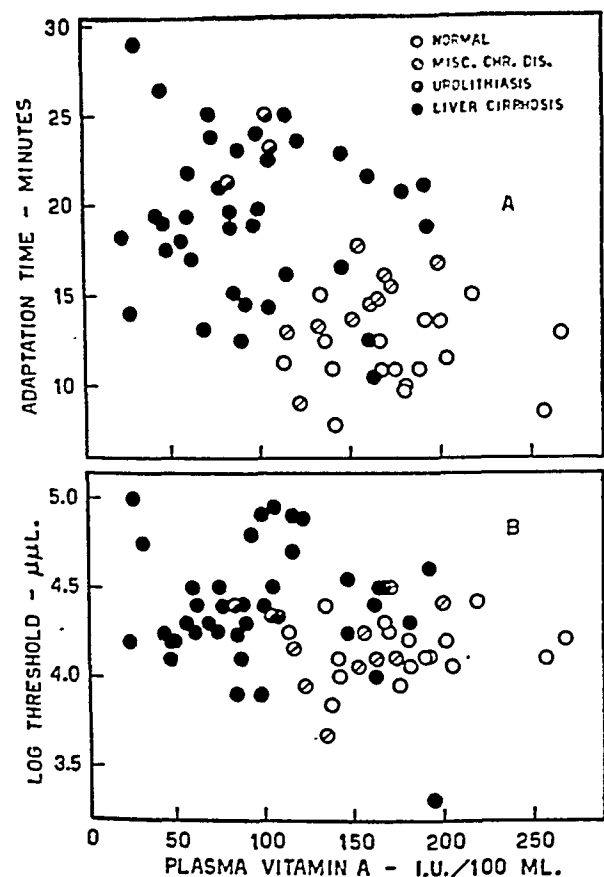


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perimentally to be an index of the amount of the vitamin stored in the liver (16, 17). Thus, the two types of measurement probably record quantitative variations in two quite different aspects of vitamin A metabolism.

SUMMARY

Measurements of dark adaptation upon 37 normal persons revealed no sex differential. In determinations of the plasma vitamin A and total carotenoid levels in 44 normal persons, the mean vitamin A level for the women was found to be 14 per cent lower than that for the men, while the mean carotenoid levels were the same in the two sexes.

Sixty-seven simultaneous dark adaptation and plasma vitamin A and carotenoid measurements were obtained in 14 normal persons, 18 persons with cirrhosis of the liver, and 7 persons with various other chronic diseases. Within the cirrhotic and normal groups, separately considered, no significant correlations were observed between the plasma vitamin A or the plasma carotenoid levels and the dark adaptation values. When all of the normal and abnormal subjects were grouped together as a single population, however, a degree of correlation between the dark adaptation measurements and the vitamin A values became apparent. This relation was interpreted as arising from differences peculiar to the several diagnostic groups studied, rather than from a causal relation between the level of vitamin A in the blood and the rate and extent of dark adaptation.

BIBLIOGRAPHY

1. Wald, G., Jeghers, H., and Arminio, J., An experiment in human dietary night blindness. *Am. J. Physiol.*, 1938, 123, 732.
2. Hecht, S., and Mandelbaum, J., Dark adaptation and experimental human vitamin A deficiency. *Am. J. Physiol.*, 1940, 130, 651.
3. Steffens, L. F., Bair, H. L., and Sheard, C., Photometric measurements on visual adaptation in normal adults on diets deficient in vitamin A. *Proc. Staff Meet., Mayo Clin.*, 1939, 14, 698.
4. Steininger, G., Roberts, L. J., and Brenner, S., Vitamin A in the blood of normal adults. *J. A. M. A.*, 1939, 113, 2381.
5. Isaacs, B. L., Jung, F. T., and Ivy, A. C., Clinical studies of vitamin A deficiency. *Arch. Ophth.*, 1940, 24, 698.
6. Jeghers, H., The degree and prevalence of vitamin A deficiency in adults. *J. A. M. A.*, 1937, 109, 756.
7. Booher, L. E., and Callison, E. C., The minimum vitamin-A requirements of normal adults. *J. Nutrition*, 1939, 18, 459.
8. Jeans, P. C., Blanchard, E. L., and Satterthwaite, F. E., Dark adaptation and vitamin A. *J. Pediat.*, 1941, 18, 170.
9. Haig, C., and Lewis, J. M., Simple method of measuring brightness threshold of dark adapted eye at all ages. *Proc. Soc. Exper. Biol. and Med.*, 1939, 41, 415.
10. Lewis, J. M., and Haig, C., Vitamin A requirements in infancy as determined by dark adaptation. *J. Pediat.*, 1939, 15, 812.
11. Haig, C., Hecht, S., and Patek, A. J., Jr., Vitamin A and rod-cone dark adaptation in cirrhosis of the liver. *Science*, 1938, 87, 534.
12. Patek, A. J., Jr., and Haig, C., The occurrence of abnormal dark adaptation and its relation to vitamin A metabolism in patients with cirrhosis of the liver. *J. Clin. Invest.*, 1939, 18, 609.
13. Zaffke, K. H., Hemeralopie als Symptom bei Thyreotoxikosen und Lebererkrankungen. *Deutsches Arch. f. klin. Med.*, 1939, 183, 433.
14. Wohl, M. G., and Feldman, J. B., Vitamin A deficiency in diseases of liver. *J. Lab. and Clin. Med.*, 1940, 25, 485.
15. Bajardi, G., and Galeone, A., La cecità notturna quale sintomo di malattia epatica. *Policlinico (sez. prat.)*, 1941, 48, 193.
16. Lewis, J. M., Bodansky, O., Falk, K. G., and McGuire, G., Relationship of vitamin A blood level in the rat to vitamin A intake and to liver storage. *Proc. Soc. Exper. Biol. and Med.*, 1941, 46, 248.
17. Horton, P. B., Murrill, W. A., and Curtis, A. C., Vitamin A and carotene. I. The determination of vitamin A in the blood and liver as an index of vitamin A nutrition of the rat. *J. Clin. Invest.*, 1941, 20, 387.
18. Haig, C., and Post, J., Vitamin A concentration in rat liver during recovery from CCl_4 cirrhosis. *Proc. Soc. Exper. Biol. and Med.*, 1941, 48, 710.
19. Lasch, F., Über den Vitamin A-Spiegel im Blute bei Leberkrankheiten. *Klin. Wchnschr.*, 1938, 17, 1107.
20. Lindqvist, T., Studien über das Vitamin A beim Menschen. *Acta med. Scand.*, 1938, Supp. 97, pp. 1-262; 1-52.
21. Rubegni, R., Il contenuto in vitamin A e in carotina del siero umano in varie condizioni patologiche. *Policlinico (sez. med.)*, 1939, 46, 565.
22. Haig, C., and Patek, A. J., Jr., Vitamin A deficiency in Laennec's cirrhosis. The relative significance of the plasma vitamin A and carotenoid levels and of the dark adaptation time. *J. Clin. Invest.*, 1942, 21, 309.
23. Moore, T., Vitamin A and carotene: The vitamin A reserve of the adult human being in health and disease. *Biochem. J.*, 1937, 31, 155.

24. Breusch, F., and Scalabrino, R., Die quantitativen Verhältnisse der Leberlipide. *Ztschr. f. d. ges. exper. Med.*, 1934, 94, 569.
25. Woo, T. T., and Chu, F. T., Vitamin A content of livers of Chinese infants, children and adults. *Chinese J. Physiol.*, 1940, 15, 83.
26. Ralli, E. P., Papper, E., Paley, K., and Bauman, E., The vitamin A and carotene content of human liver in normal and in diseased subjects. *Arch. Int. Med.*, 1941, 68, 102.
27. Hecht, S., and Schlaer, S., An adaptometer for measuring human dark adaptation. *J. Optic. Soc. America*, 1938, 28, 269.
28. Haig, C., The course of rod dark adaptation as influenced by the intensity and duration of pre-adaptation to light. *J. Gen. Physiol.*, 1941, 24, 736.
29. Kimble, M. S., The photolorimetric determination of vitamin A and carotene in human plasma. *J. Lab. and Clin. Med.*, 1939, 24, 1055.
30. Daniel, E. P., and Munsell, H. E., Vitamin A Content of Foods. U. S. Dept. of Agric. misc. publ. 275, June, 1937.
31. Catel, W., Klinische und tierexperimentelle Studien über die normale und pathologische Physiologie des A-Vitamins. *Monatschr. f. Kinderh.*, 1938, 73, 316.
32. Murrill, W. A., Horton, P. B., Leiberman, E., and Newburgh, L. H., Vitamin A and carotene. II. Vitamin A and carotene metabolism in diabetics and normals. *J. Clin. Invest.*, 1941, 20, 395.
33. Lewis, J. M., Bodansky, O., and Haig, C., Level of vitamin A in the blood as an index of vitamin A deficiency in infants and in children. *Am. J. Dis. Child.*, 1941, 62, 1129.
34. Pett, L. B., and LePage, G. A., Vitamin A deficiency. III. Blood analysis correlated with a visual test. *J. Biol. Chem.*, 1940, 132, 585.
35. Josephs, H. W., Baber, M., and Conn, H., Studies in vitamin A. *Bull. Johns Hopkins Hosp.*, 1941, 68, 375.
36. Lewis, J. M., Bodansky, O., Falk, K. G., and McGuire, G., Vitamin A requirements in the rat. *J. Nutrition*, 1942, 23, 351.
37. Patek, A. J., Jr., and Haig, C., Effect of administration of thyroid extract and of α -dinitrophenol upon dark adaptation. *Proc. Soc. Exper. Biol. and Med.*, 1941, 46, 180.

CIRRHOSIS OF THE LIVER AMONG RATS RECEIVING DIETS POOR IN PROTEIN AND RICH IN FAT¹

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The importance of fat deposition in the liver as a possible contributory factor in the pathogenesis of cirrhosis was emphasized by Connor in 1939 (1). Best and Ridout (2) found that a high fat, low protein, choline-poor diet, when administered to rats, produced extensive fat deposition in the liver. It therefore seemed reasonable to administer such a diet to rats over an extended period of time in order to determine whether cirrhosis might ultimately ensue. Experiments to this end have been carried out in this laboratory (3), and recent reports of similar independent investigations have been made by György and Goldblatt (4), by Blumberg and McCollum (5), and by Lillie, Daft, and Sebrell (6). Also Chaikoff and Connor have recently reported the occurrence of cirrhosis of the liver in dogs receiving a high fat diet (7).

That fatty changes in the liver may be induced or aggravated in the rat by cystine feeding has been shown by Curtis and Newburgh (8), and by Lillie (9). More recently Earle and Victor (10) have reported that prolonged cystine feeding leads ultimately to cirrhosis. Cholesterol has long been known to increase the lipid content of the liver (2) and cirrhosis in the rabbit after cholesterol administration has also been observed by Chalatow (11), and more recently by Leary (12). Choline, betaine, and related compounds are agents which protect against the development of fatty livers induced by a variety of causes (2). It has also been shown by Griffith and Wade (13) that choline exerts an ameliorating effect upon the kidney necrosis which follows the administration of cystine. Because of the above findings additional groups of rats were given cystine, cholesterol, and betaine, in order to observe any modifying influence which these compounds might exert on the development of liver lesions.

¹ This work was made possible by a grant in aid from the Howard M. Hanna, III, Memorial Fund.

METHODS

White rats of the Sprague Dawley strain were kept in individual cages and allowed to consume the proffered diet as desired. A basic diet, high in fat and low in protein and choline, was prepared of the following constituents per 100 grams: rice starch, 44 grams; casein, 8 grams; hydrogenated cottonseed oil, 38 grams; cod liver oil, 2 grams; salt mixture, 5 grams; whole dried brewer's yeast, 3 grams (equivalent to a daily intake of approximately 0.25 grams).² Measurement of the quantity of diet consumed by each rat was made.

Early in this work it was discovered that cirrhotic lesions of maximum severity could be produced in mature rats weighing more than 250 grams. Hence the experiments which are reported at this time were performed on rats weighing between 250 and 450 grams and ranging in age from 4 to 9 months when they were first given the diet. There were a few exceptions which may be noted in Table I. Males seemed to be more susceptible to cirrhotic changes than females (Table I) and therefore they were used exclusively in all experiments except those employing basal diet alone. In another preliminary study, not recorded below, a number of rats were killed at varying intervals after being placed on the basal diet, in order to determine the time of onset of cirrhosis. The first evidence of fibrosis was detectable not earlier than the fourth month after the start of the diet. For this reason all rats included here were killed at the end of 150 days. Any animals dying before this time were excluded from the protocols.

RESULTS

Basal diet alone

With varying severity, the following picture was seen. The livers were large with roughened surface in hobnail form. No ascites or jaundice was observed. Those rats having the largest livers had the largest spleens. Moderate hypochromic anemia was present. The kidneys were

² The casein was Labco brand, alcohol extracted "vitamin free," and the yeast was Strain G of Anheuser-Busch. The salt mixture was No. 185 of McCollum and Simmonds (14) with the addition of sodium fluoride 0.036 parts, manganese sulfate 0.018 parts, and potassium iodide 0.90 parts per thousand of salt mixture.

large, pale, and coarsely granular in surface. Microscopically, the liver exhibited multilobular increases in periportal connective tissue, fat infiltration, and occasional collections of lymphocyte-like cells in the periportal tissues. Bile ducts were prominent, and occasionally focal areas of necrosis were seen with lysis of cells, pyknotic nuclei, and hemorrhage. In the kidneys there were areas of cortical necrosis with hemorrhage, scarring, and focal necrotizing nephrosis. Except in the severely scarred areas, lesions in the glomeruli were minimal. The animals with relatively more severe hepatic lesions tended to have relatively less severe renal lesions and the reverse. Examination of the kidneys of a number of rats used in preliminary experiments indicated that the renal lesions were independent of age or sex. A summary of the observations made upon this group of rats is presented in Table I.

Modifications of the basal diet

Groups of 10 rats each were given the basal diet modified in various ways. With thiamin chloride and riboflavin added to the diet, 20 gamma

of each per rat per day, the results were essentially the same as with basal diet alone. Addition of brewer's yeast prevented the necrosis and fibrosis when given in quantities of 2 grams per rat daily, but under these circumstances the dietary can no longer be considered to be low in protein or choline, and the proportion of fat is relatively reduced. Likewise, substitution of 2 grams of molasses³ daily for a part of the carbohydrate resulted in prevention of the necrotic and cirrhotic lesions. Variation of carbohydrate source from rice starch to glucose or sucrose, and of fat source to beef dripping or pig lard, did not alter the results.

Effect of varying the percentage of protein and of fat

The casein content of the original diet was increased to 25 per cent at the expense of the carbohydrate fraction. Only one of the 10 animals in this group showed increase in periportal connective tissue, and that was of very minor de-

³ Trixy molasses manufactured by D. B. Scully Syrup Company.

TABLE I
Rats receiving basal diet
MALES

Rat number	Initial body weight	Body weight at death	Liver weight	Spleen weight	Kidney		Liver changes			Daily food intake
					Weight	Lesions	Fat increase	Necrosis	Cirrhosis	
	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>					<i>grams</i>
1	555	290	12.7	0.58	1.8	Moderate	Slight	Slight	Slight	8.6
2	365	301	13.3	0.60	1.9	Moderate	Moderate	Slight	Slight	7.1
3	310	269	14.7	0.71	1.6	Slight	Moderate	Slight	Moderate	7.8
4	380	284	10.0	0.58	2.0	Slight	Slight	Moderate	Slight	8.3
5	392	334	11.8	0.70	2.0	Moderate	Moderate	Slight	Moderate	9.6
6	345	298	7.3	0.70	1.9	Moderate	Slight	Slight	Slight	9.1
7	260	211	9.3	0.40	1.4	Slight	Moderate	Slight	None	7.3
8	165	175	8.2	0.50	1.5	Slight	Moderate	Slight	None	8.5
9	210	152	15.4	0.60	1.2	Slight	Moderate	None	Moderate	5.1
10	480	323	9.4	0.61	2.6	Moderate	Slight	Slight	Slight	7.4

FEMALES

1	202	169	8.0	0.48	1.5	Moderate	Moderate	None	None	5.0
2	225	201	8.1	0.74	1.7	Moderate	Moderate	Moderate	Moderate	5.8
3	241	214	8.1	0.58	1.1	Moderate	Slight	None	None	5.5
4	230	242	9.3	0.60			Moderate	Slight	Slight	6.1
5	215	171	7.2	0.48	1.6	Moderate	Moderate	Slight	Slight	4.8
6			Died of pneumonia after 67 days.					Tissues macerated.		
7	200	181	7.9	0.55			Moderate	None	Slight	5.2
8	190	178	7.8	0.49			Slight	None	None	5.0
9	200	180	8.1	0.48			Slight	None	None	5.1
10	135	145	10.6	0.49	1.0	Moderate	Moderate	Moderate	Slight	5.8

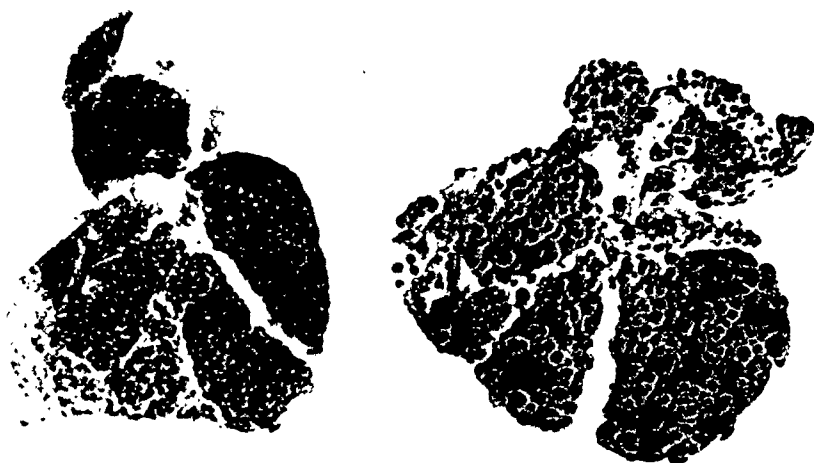


FIG. 1. GROSS ASPECT OF CIRRHOTIC LIVERS

On the left, from an animal receiving the basal diet; on the right, from an animal receiving basal diet plus 10 mgm. of L-cystine daily.

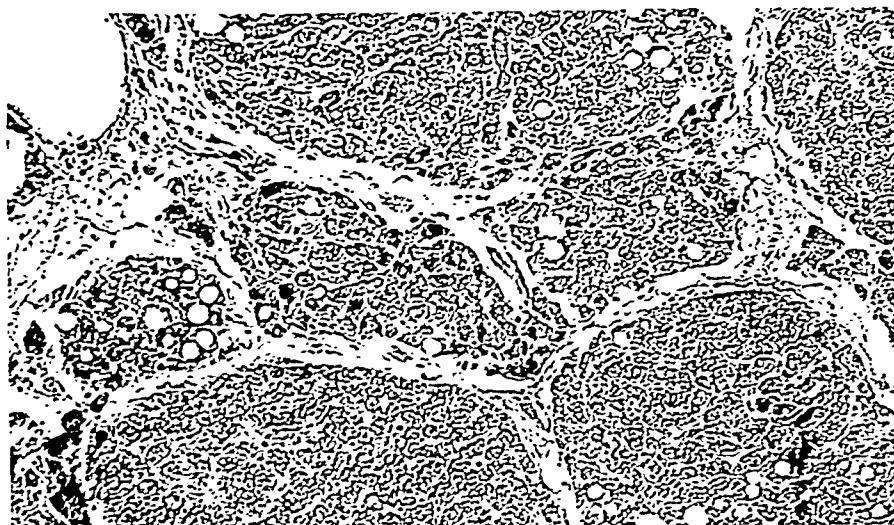


FIG. 2. SECTION OF LIVER FROM ANIMAL RECEIVING BASAL DIET

(Hematoxylin-eosin) $\times 140$



FIG. 3. SECTION OF LIVER FROM ANIMAL RECEIVING BASAL DIET TO SHOW FAT INFILTRATION

(Mallory's connective tissue stain) $\times 140$

gree. The fat content of their livers was considerably reduced, and renal disease was slight. Increase of the protein content to 25 per cent by the addition of 17 per cent gelatin to the original diet, at the expense of carbohydrate, had the same result when given to a group of 10 rats as increase in casein, except that fat infiltration was not as greatly reduced.

A decrease in fat content to 10 per cent, with increase in carbohydrate, resulted in some decrease in severity of the liver disease. Four of the 5 rats in this group showed minimal cirrhosis and one showed none. Renal lesions were also much less severe than in the original group.

Effect of betaine

The addition of betaine hydrochloride to the original diet, a choline analogue, in doses of 50 mgm. per rat daily, diminished the severity of the liver lesions, not as effectively as increased protein, but more so than diminished fat. The renal lesions were less severe than in rats receiving the basal diet. Two of the 10 animals in this group failed to survive until the end of the experiment and were excluded from the tabulation in Table II.

Addition of cystine

The addition of 1-cystine, 10 mgm. per rat daily, aggravated the fibrotic lesions of the liver, and, in this group, there was the same marked variation in severity of cirrhosis as seen in the

original group. In those animals which were affected, however, there was a tendency for the increase in periportal tissue to be more extensive than in those on the basal diet. The livers of these rats were larger than those of the original group and showed a surface covered with rounded nodules. Histologically, large whorls of regenerating liver tissue were seen and among them some multinucleate giant cells. The renal lesions in this group were relatively mild except in the case of one rat which exhibited very severe scarring. Two rats had ascites but no jaundice was observed. In 2 animals, neoplasms were found. One was a primary cancer of the right lung with metastases to the mediastinal nodes. It consisted of nests and cords of small round cells of varying sizes and shapes, with frequent mitoses and moderately rich connective tissue stroma. The surrounding lung tissue was compressed but no distinct capsule could be made out. The other neoplasm arose in the pancreas and consisted of tissue similar to that described above, with many mitotic figures, multinucleate cells, and widespread metastases. The 2 rats which suffered from neoplasms had relatively less severe renal and hepatic lesions than others in this group. One animal failed to survive the 150 day experimental period.

Addition of betaine and cystine

Addition of 50 mgm. of betaine hydrochloride to the diet containing 1-cystine prevented the very severe cirrhotic changes produced by cystine alone.

TABLE II

Type of diet	Number of rats	Daily food consumption	Initial body weight	Final body weight	Liver weight	Spleen weight	Kidney weight	Number with kidney lesions				Number with liver lesions											
												Increased fat				Necrosis				Cirrhosis			
								None	Slight	Moderate	Severe	None	Slight	Moderate	Severe	None	Slight	Moderate	Severe	None	Slight	Moderate	Severe
Basal.....	10*	grams	grams	grams	grams	grams	grams	0	5	5	0	0	4	6	0	1	8	1	0	2	5	3	0
25 per cent casein ..	10	8.2	368	346	11.4	1.3	2.8	0	9	1	0	1	8	1	0	9	1	0	0	9	1	0	0
10 per cent fat.....	5		263	195	6.8	0.5	1.5	1	4	0	0	2	0	3	0	4	1	0	0	1	4	0	0
Betaine.....	8		270	183	6.8	0.5	1.6	3	5	0	0	0	8	0	0	4	3	1	0	6	2	0	0
Cystine.....	9		332	233	12.7	0.8	2.4	0	7	1	1	0	3	6	0	9	0	0	0	2	1	5	0
Betaine and cystine	9		334	217	11.0	0.8	1.9	9	0	0	0	0	2	7	0	7	1	1	0	0	9	0	0
Cholesterol.....	5		346	233	20.2	1.3	2.7	0	0	3	2	0	0	0	5	5	0	0	0	0	2	0	3

* Only male rats are included.



FIG. 4. SECTION OF KIDNEY FROM RAT RECEIVING BASAL DIET (Hematoxylin-eosin) \times 140

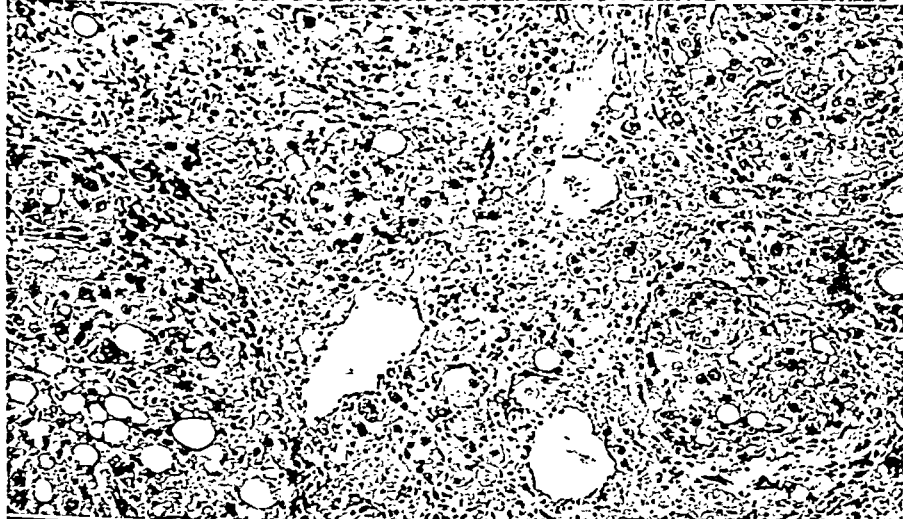


FIG. 5. SECTION OF LIVER FROM RAT RECEIVING BASAL DIET PLUS 1-CYSTINE (Hematoxylin-eosin) \times 135



FIG. 6. SECTION OF KIDNEY FROM ANIMAL RECEIVING BASAL DIET PLUS 1-CYSTINE (Hematoxylin-eosin) \times 175

Renal lesions were absent in this group. Two animals showed nodular structures in the liver, about 1 cm. in diameter, which appeared to be hepatomas. They were surrounded by an ill-defined capsule of compressed liver tissue and were made up of multiple foci of cellular cords and clumps having no specific architectural arrangement. The clumps consisted of cells with nuclei arranged peripherally to the center at which there appeared to be a lumen. There were numerous mitotic figures and multinucleate cells. No metastases were observed. Blood supply in these areas was rich, and the cells contained very little fat as compared to surrounding liver cells. One animal of this group failed to survive.

Addition of cholesterol

Two per cent cholesterol added to the original diet caused the most severe cirrhosis and the most uniform distribution of cirrhosis among all the various groups of animals. It also caused the most severe fat infiltration of the livers although necrosis was absent. The kidneys in this group showed very severe tubular necrosis and hemorrhage.

DISCUSSION

It would seem reasonable to assume that the hepatic cirrhosis encountered in the present experiments may be attributed primarily to parenchymal damage, and that an elucidation of the pathogenesis of the lesion would necessitate an explanation of the cause of the hepatic cell dissolution. Protoplasm is undoubtedly subject to a constant state of equilibrium between protein synthesis and proteolysis. Any relative decrease in the former or increase in the latter could lead to a cellular disintegration which morphologically would consist of necrosis or atrophy. Of the various body tissues which have been studied, the highest rates of intracellular proteolysis have been found in liver and kidney (15). The predilection of the present lesions for liver and kidney is therefore correlated with the susceptibility of the cells of these organs to autolysis. That a diet deficient in protein would promote autolysis seems quite reasonable inasmuch as the supply of protein would be insufficient to support the counterbalancing reaction of synthesis. The particular tend-

ency of the liver to lose protein during fasting is brought out by the experiments of Addis, Poo, and Lew (16). The protective action of high protein diets against liver damage by chloroform (17) is further evidence of the importance of an adequate protein supply in the maintenance of liver cell integrity.

Activation of proteolysis in liver hash has been extensively studied and it has been shown that this may be brought about by a number of means (15, 18). Briefly summarized, these are: the addition of SH containing compounds, of some heavy metals, chiefly arsenic and manganese, of fats, of phosphorus, of some halides; and by anoxia. It is therefore of interest to find that cirrhosis has been aggravated in the above experiments by high cystine and fat dietaries, and has been produced in experimental animals by arsenic, manganese, phosphorus, and organic halides (carbon tetrachloride and chloroform) (19).

Phenylhydrazine is known to produce cirrhosis (19) and is also known to inhibit oxygen uptake by liver slices (20). With inhibition of oxygen intake, the equivalent of a state of anoxia might exist and such a state would accelerate proteolysis.

The protection offered by molasses against the liver cirrhosis cannot be adequately interpreted at this time, inasmuch as this product is a highly complex mixture of plant extractives.

With regard to the above described neoplasms, the evidence is not conclusive that they were more than isolated spontaneous occurrences in old animals that happened to be on the high cystine diets. This, however, seems improbable because 4 neoplasms occurred in 20 rats receiving cystine plus basal diet, whereas in 200 other rats of the same age, strain, and weight receiving other diets in these experiments, no neoplasms were observed. An etiological relationship between cirrhosis of the liver and hepatic neoplasms is suggested by the almost invariable coexistence of cirrhosis in the presence of primary liver tumors in man. Acceleration of the growth rate of pre-existing neoplasms in animals receiving high cystine dietaries has been reported by Voegtlin (21) and related by him to his theories of the reversibility of protein destruction in new growth and hypertrophy (22).

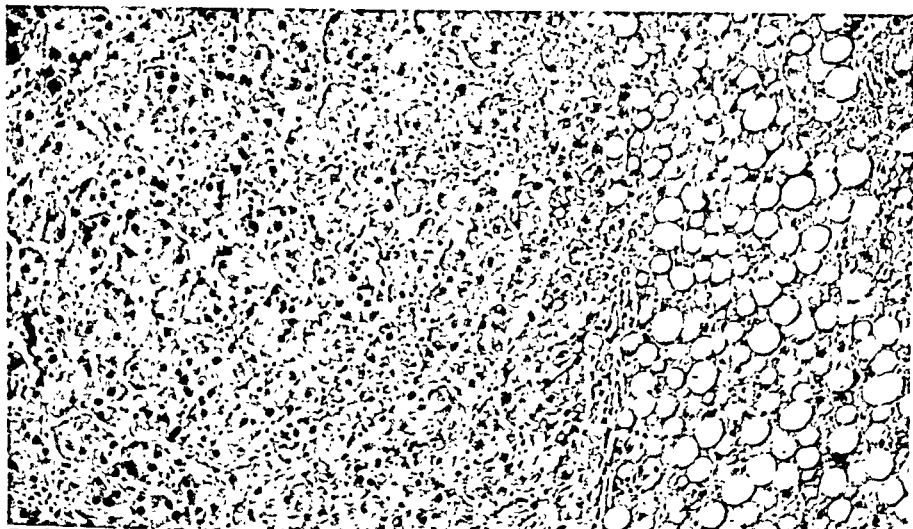


FIG. 7. HEPATOMA
(Hematoxylin-eosin) \times
140

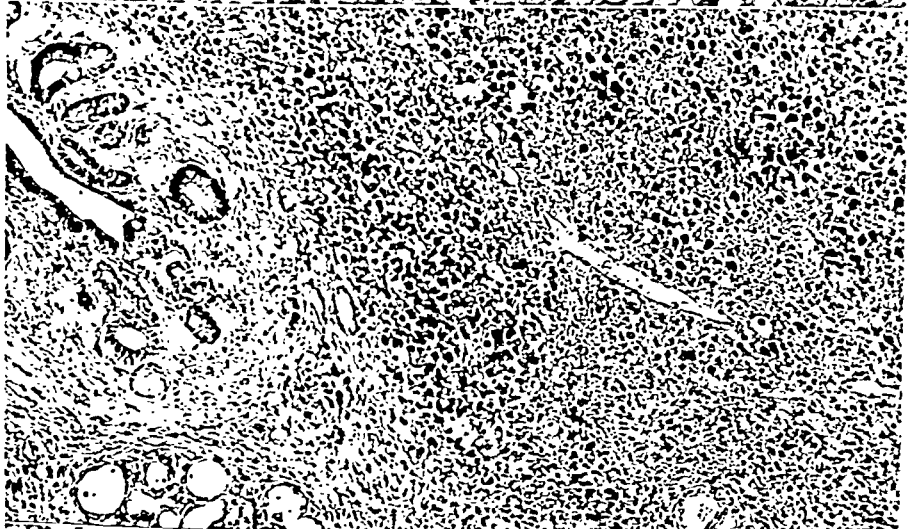


FIG. 8. SECTION OF
CARCINOMA OF THE PAN-
CREAS INVADING THE
WALL OF THE DUODENUM
(Hematoxylin-eosin) \times
135

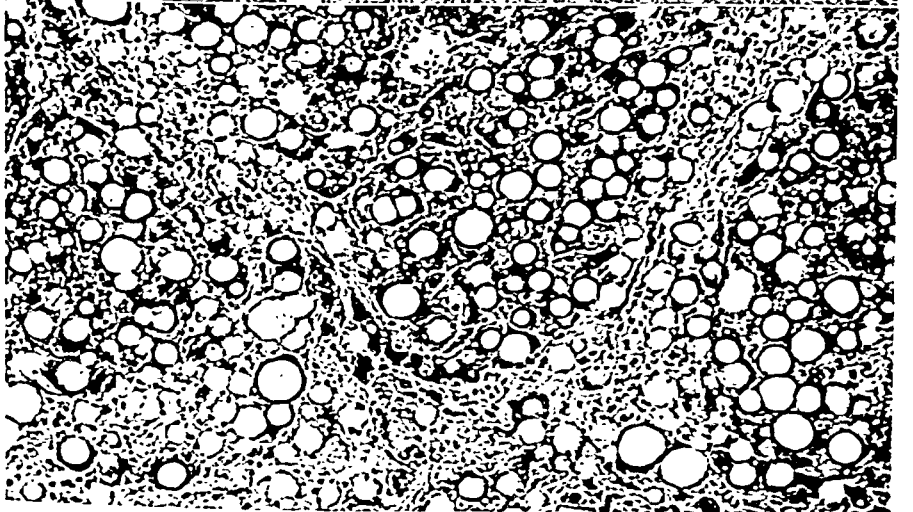


FIG. 9. SECTION OF
LIVER FROM RAT RECEIV-
ING BASAL DIET PLUS
CHOLESTEROL
(Hematoxylin-eosin) \times
140

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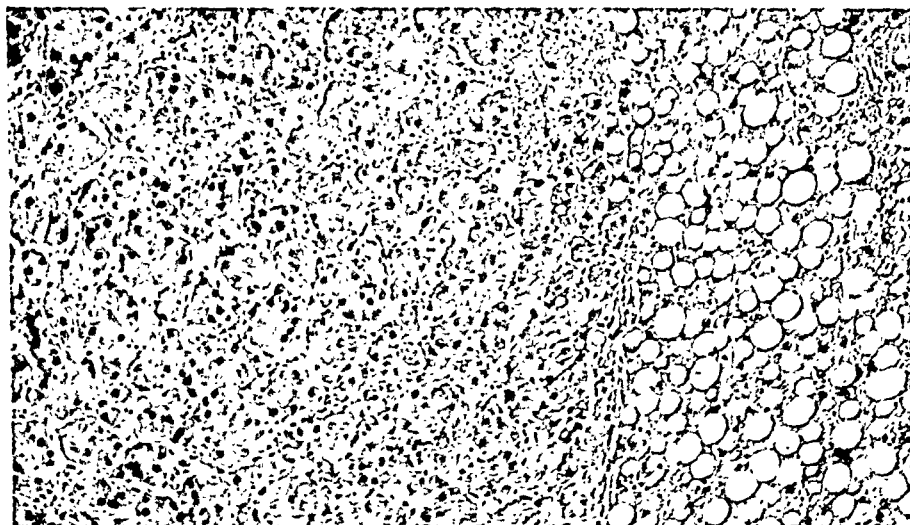


FIG. 7. HEPATOMA
(Hematoxylin-eosin)
140

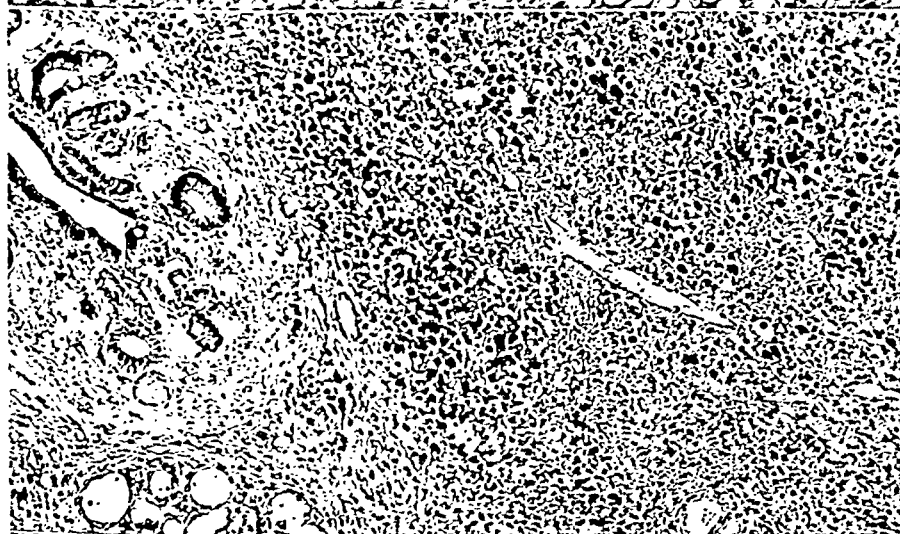


FIG. 8. SECTION
CARCINOMA OF THE P
CREAS INVADING
WALL OF THE DUODE
(Hematoxylin-eosin)
135

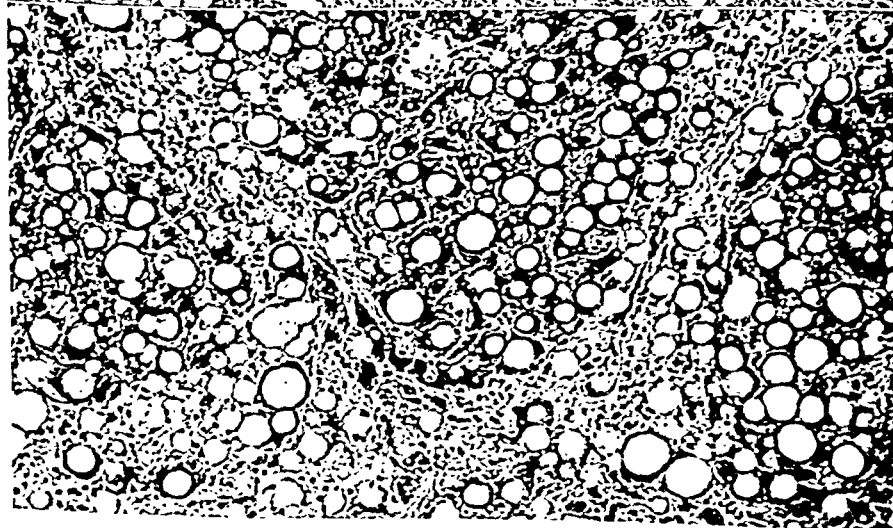


FIG. 9. SECTION
LIVER FROM RAT REC
ING BASAL DIET P
CHOLESTEROL
(Hematoxylin-eosin)
140

SUMMARY

A description has been given of necrosis and cirrhosis of the liver, and of renal necrosis, fibrosis, and hemorrhage, among rats receiving diets poor in protein and choline, and rich in fat.

The hepatic lesions were prevented by an increase in the protein content of the diet and by the addition of molasses. A reduction in the fat content diminished the severity of the lesions as did the addition of betaine. Cystine and cholesterol increased the severity of the fibrotic changes. The effect of cystine was ameliorated by betaine. Thiamin and riboflavin were without influence on the disease. Yeast prevented the lesions but its efficacy could be due to the extra protein and choline which it contributed.

The renal lesions, like those of the liver, were prevented by brewer's yeast and molasses. Increased protein intake materially reduced the severity of the lesions, and thiamin and riboflavin again were without effect. A reduced proportion of fat in the diet, and the addition of betaine to the basal diet, decreased the severity of the lesions. Cystine alone had no effect on the lesions, although rats receiving cystine plus betaine showed no detectible kidney disease. Cholesterol exaggerated the lesions to a marked degree.

Neoplasms occurring in 20 per cent of the rats receiving added cystine are described.

BIBLIOGRAPHY

1. Connor, C. L., The etiology and pathogenesis of alcoholic cirrhosis of the liver. *J. A. M. A.*, 1939, 112, 387.
2. Best, C. H., and Ridout, J. H., Choline as a dietary factor. *Ann. Rev. Biochem.*, 1939, 8, 349.
- 3a. Webster, G. T., *Trans. Assn. Am. Phys.*, 1940, 55, 139.
- b. Webster, G. T., Dietary liver disease in rats. *J. Clin. Invest.*, 1941, 20, 440.
4. György, P., and Goldblatt, H., Experimental production of dietary liver injury in rats. *Proc. Soc. Exper. Biol. and Med.*, 1941, 46, 492.
5. Blumberg, H., and McCollum, E. V., The prevention by choline of liver cirrhosis in rats on high fat low protein diets. *Science*, 1941, 93, 598.
6. Lillie, R. D., Daft, F. S., and Sebrell, W. H., Jr., Cirrhosis of the liver in rats on a deficient diet and the effect of alcohol. *Pub. Health Rep.*, 1941, 56, 1255.
7. Chaikoff, I. L., and Connor, C. L., Production of cirrhosis of liver of normal dog by high fat diets. *Proc. Soc. Exper. Biol. and Med.*, 1940, 43, 638.
8. Curtis, A. C., and Newburgh, L. H., The toxic action of cystine on the liver of the albino rat. *Arch. Int. Med.*, 1927, 39, 828.
9. Lillie, R. D., Histo-pathologic changes produced in rats by the addition to the diet of various amino acids. *Pub. Health Rep.*, 1932, 47, 83.
10. Earle, D. P., Jr., and Victor, J., Cirrhosis of the liver caused by excess dietary cystine. *J. Exper. Med.*, 1941, 73, 161.
11. Chalataw, S. S., Über experimentelle Cholesterin-Lebercirrhose in Verbindung mit eigenen neuen Erhebungen über flüssige Kristalle des Organismus und über den Umbau der Leber. *Beitr. Path. Anat.*, 1914, 57, 85.
12. Leary, T., The genesis of atherosclerosis. *Arch. Path.*, 1941, 32, 507.
13. Griffith, W. H., and Wade, N. J., Relation of methionine, cystine, and choline to renal lesions occurring on low choline diets. *Proc. Soc. Exper. Biol. and Med.*, 1939, 41, 333.
14. McCollum, E. V., and Simmonds, N., A study of the dietary essential water soluble B in relation to its solubility and stability towards reagents. *J. Biol. Chem.*, 1918, 33, 55.
15. Bradley, H. C., Autolysis and atrophy. *Physiol. Rev.*, 1922, 2, 415.
16. Addis, T., Poo, L. J., and Lew, W., Protein loss from liver during a two day fast. *J. Biol. Chem.*, 1936, 115, 117.
17. Goldschmidt, S., Vars, H. M., and Ravdin, I. S., The influence of the foodstuffs upon susceptibility of the liver to injury by chloroform, and the probable mechanism of their action. *J. Clin. Invest.*, 1939, 18, 277.
18. Bradley, H. C., Autolysis and atrophy. *Physiol. Rev.*, 1938, 18, 173.
19. Moon, V. H., *Compt. rend. deux Conf. Internat. de Path. Geograph.*, A. Oosthoek, Utrecht., 1934, page 606.
20. Bernheim, F., Bernheim, M. L. C., and Michel, H. O., The action of p-aminophenol on certain tissue oxidations. *J. Pharmacol. and Exper. Therap.*, 1937, 61, 311.
21. Voegtlin, C., Johnson, J. M., and Thompson, J. W., Glutathione and malignant growth. *Pub. Health Rep.*, 1936, 51, 1689.
22. Voegtlin, C., Observations concerning the chemistry of cell growth and division. *Cold Spring Harbor Symp., Quant. Biol.*, 1934, 2, 84.

ON THE OCCURRENCE OF DYSPNEA, DIZZINESS AND PRECORDIAL DISTRESS OCCASIONED BY THE POOLING OF BLOOD IN VARICOSE VEINS

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Varicose veins of the legs are known to have local secondary effects such as edema of the leg, eczema of the skin, ulceration and even hemorrhage. Sequelae such as thrombosis and phlebitis of the enlarged veins may in turn cause pulmonary emboli with their distressing pulmonocardiac symptoms. However, we can find no clear evidence in the medical literature that the pooling of blood in varicose veins leads to a decrease in the efficiency of cardiac and circulatory function.¹ It is our intention to present here evidence, both clinical and physiological, indicating that such a form of circulatory embarrassment occurs in varying degrees in persons who have varicose veins.

The failure in the past to recognize this type of decrease in circulatory function may be due in part to the fact that the symptoms are usually moderate in nature and seldom lead to actual cardiac failure. However, fatigue, dyspnea, dizziness, fainting and precordial distress are symptoms that may trouble the patient in whom the heart is burdened to maintain its compensation. These symptoms should be recognized as possibly being the effect of pooling of blood in varicose veins. Surgical treatment of such patients should restore their hemodynamics toward normal and thus bring relief from their symptoms, provided that the heart has not been permanently damaged. In the aged and in those with known heart disease,

the added burden from varicose veins is enough to provoke severe symptoms.

The erect posture imposes a certain load on the circulatory system even in normal individuals. The increased hydrostatic pressure in the veins in the lower parts of the body causes a distention of blood vessels and a local accumulation of blood. Asmussen, Christensen and Nielsen (3) estimated that in normal persons standing 100 cc. of blood or even more accumulate in the lower extremities. Consequent to such stagnation a smaller amount of blood is available in the central veins to flow back into the heart; this decrease in venous return lessens the filling of the heart and results in a decrease in the cardiac output when standing. A fall in blood pressure under these conditions may be followed by a sudden increase in pressure which is probably accomplished via the baroreceptor sensitive zones in the aortic arch and the carotid sinus and also by a compensatory contraction of the blood vessels in the abdominal viscera. A decrease in the cardiac output of normal individuals has been observed by Asmussen, Grollman (4), and Sweeney and Mayer. In fact, two normal subjects of Schneerson and Crampton (6) became dizzy on quiet standing for fifteen minutes and one fainted.

The idea that varicose veins could cause dyspnea, dizziness and precordial distress has been noted in the practice of medicine when one of us was consulted by a middle-aged woman in good health who complained of attacks of severe precordial pain on walking and sometimes on standing. The clinical story suggested a coronary heart disease but careful examination of the heart disclosed no evidence of disease. The roentgenogram and the electrocardiogram of her heart were normal. The observation that large varicose veins suddenly led to the symptoms of pooling in these veins might have caused

¹ Near the completion of this work we discovered in the lectures of John Gay in 1867 (1) a description of a varicose old lady who described a correspondence between the venous distention and her dyspnea. After surgical treatment her dyspnea was much relieved. Likewise, in November 1940, Lee and Freeman (2) described circulatory disturbances produced by extensive angiomas of the lower extremities associated with varicose veins. In one of their three cases, attacks of fainting on standing were prevented by the wearing of an elastic stocking and finally saphenous ligation brought permanent relief of symptoms.

decrease in venous return to the heart that inadequate filling of the heart resulted in a deficient coronary blood flow and so caused pain. Relief from this patient's symptoms was obtained by having her wear elastic stockings on both legs.

With this experience in mind, we next interviewed a large number of persons with varicose veins of sufficient size to allow considerable venous pooling. Two hundred and fifty patients in the Out-Patient Department of the Massachusetts General Hospital, so afflicted, were questioned. The surprising result of this was to find that 47 (18 per cent) of the 250 complained of undue shortness of breath that was relieved in the recumbent position; 19 of these 47 also suffered mild precordial pain, palpitation, or were uncomfortably aware of their heart action, and 3 were women who experienced attacks of sudden dyspnea, dizziness and precordial pain, simply on standing. These 47 patients were without gross signs of the known types of heart disease, although in some the blood pressure was slightly elevated.

With this background in clinical evidence, we next turned to the laboratory for data that might explain these symptoms and also the changes that occur in the circulation of persons with large varicose veins.

METHODS

In this investigation we had planned to observe 12 normal persons and 24 patients with varicose veins, 12 of whom had symptoms such as described here and then repeat our studies after operative procedures to obliterate the venous reservoirs. However, certain practical considerations, including the sudden return of one of us (E. A.) to Denmark, have allowed us to complete our observations on only 7 normal and 12 varicose subjects. Of these 12, 5 had the symptoms mentioned; 7 returned for study after operation and 4 of these were with symptoms.

We first obtained normal, untrained subjects and confirmed the previous observations of Grollman, Asmussen, and Mayerson that the change from the recumbent to the upright position causes a decrease in the cardiac output in the tilted position. Conversely, Schneider and Crampton observed an increase in the cardiac output in changing from quiet standing to the recumbent position. Seven normal subjects were taken to the laboratory in the morning in a fasting state and placed in a recumbent position on a tilt table. After a rest period, the basal metabolic rate, vital capacity, pulse rate and blood pressure were measured. An estimation of the arteriovenous oxygen difference was made by the Grollman acetylene

method (taking the samples at intervals of 18 and 23 seconds), and the cardiac output was computed from the basal oxygen consumption and the A-V oxygen difference. The subject then was tilted passively to a 45° angle and the observations were repeated. In most subjects an electrocardiogram was taken in both positions.

Twelve patients with rather large, untreated varicose veins were then studied; 5 of these 12 had the symptoms mentioned before, chiefly dyspnea and dizziness, and 1 woman described particularly severe attacks of shortness of breath, dizziness and fainting on getting suddenly out of bed.

Finally, 3 to 17 months after these patients had had high saphenous ligations and injections of sclerosing solutions for their varicose veins, we succeeded in getting 7 of them back to the laboratory and repeated our observations. The upper photographs of Figure 1 show the subject P. D. with large varicose veins; the lower photographs show his legs 6 months after this treatment.

RESULTS AND DISCUSSION

In our patients with varicose veins it is evident that an amount larger than 500 cc. of blood accumulates in their veins during standing and walking. Consequently, the same physiologic responses could be expected but in a greater order of magnitude. We likewise observed this decrease in the cardiac output in the tilted position of our normal but untrained subjects. The accompanying data best illustrate these responses. We did not undertake to estimate the amount of blood in the veins of the legs for several reasons. First, plethysmographic methods necessitating pressure about the thigh would necessarily introduce an error, and second, the approximate knowledge of the amount pooled in no way explains the physiologic response; it only indicates the degree of the response.

Figure 2 shows that the 7 normal subjects were practically the same age as the patients and that our results agree satisfactorily with the normal values established by Grollman and by Asmussen, Christensen and Nielsen. The cardiac index (cardiac output per square meter of body surface) is not only constant under fixed conditions but like the basal metabolism is predictable, according to Grollman, for normal individuals. It will be noted that the cardiac index and the stroke volume² are higher than normal when subjects with varicose

² The stroke volume is the amount of blood ejected with each systole and its value multiplied by the pulse rate gives the cardiac output in liters per minute.



FIG. 1. SUBJECT P. D. WITH LARGE VARICOSE VEINS; THE LOWER PHOTOGRAPHS SHOW HIS LEGS 6 MONTHS AFTER SAPHENOUS LIGATION AND INJECTION

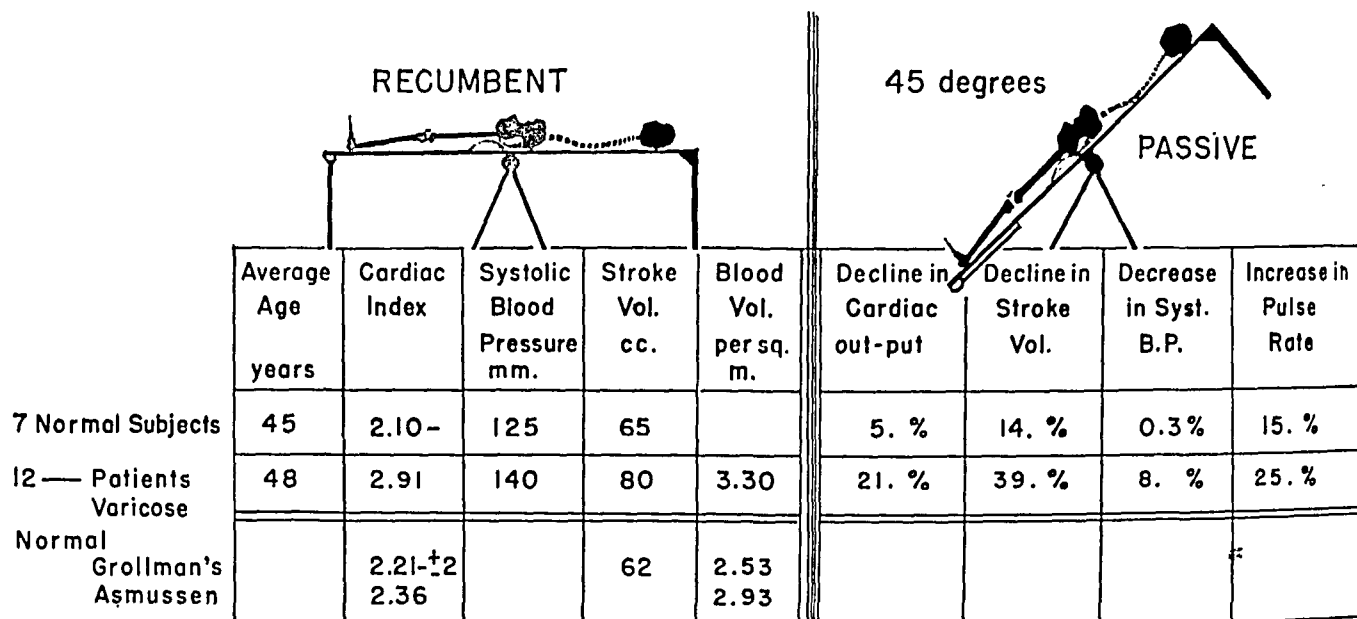


FIG. 2. CIRCULATORY FUNCTIONS OF NORMAL SUBJECTS COMPARED WITH PATIENTS WITH VARICOSE VEINS

veins are recumbent. *The reason for this may be that with varices there is a greater blood volume and so in the recumbent position a greater amount of blood returns to the heart. This is suggested by the high average blood volume of 3.30 liters per square meter of body surface, compared with the normals established by Gibson and Evans (7) using the same technique. Their values for normal males were 2.93 and for females 2.53. Our high value indicates an added load in the circulation.*

From Figure 2 it is also evident that on tilting these subjects and filling their varicose veins the cardiac output fell about four times as much as it did in the normal subjects and the systolic blood pressure was somewhat lowered but never enough to suggest a postural hypotension. *Possibly it is this comparatively great fall from a higher than normal level of cardiac output which causes the symptoms described here.* Kerr (8) described relief of dyspnea and anginal pain by having his patients with a pendulous abdomen wear an abdominal belt. The situation may be analogous with the pooling of blood in the legs in one case, and in the abdominal cavity in the other. When tilted, the average cardiac indices in the normal persons and in those with varicose veins, both before and after operation, were still within the normal range (Table I). However, analysis of individual responses shows that in 4 of 6 patients the cardiac output in the tilted

position is definitely lowered after the treatment for the varicose veins. This further supports the explanation that an actual reduction of the load in the circulation was accomplished by removal of the venous reservoir, and could explain the clinical fact that many patients note fewer symptoms of fatigue after the obliteration of their engorged veins.

Figure 3 shows the measurements of circulatory functions that were made several months after corrective operations for the varicosities of 7 subjects. In all but 1 subject (F. B.) high cardiac indices had been present and after removal of their venous reservoir this value became normal in 2 and lower in 2; also in 6 of the 7, the blood pressure when recumbent was lowered. Likewise the decreases in stroke volume and blood pressure on tilting became much less in all except 2 subjects. From the last column it can be noted that the increase in pulse rate on tilting became normal in all but these same 2 after operation. These 2 subjects deserve particular comment. M. MacD. had symptoms of dyspnea and dizziness and often fainted if she got out of bed and stood up suddenly. Following saphenous ligations she had no further such attacks and the decline in stroke volume on tilting was less. However, moderate varices in her right leg persist. The other subject, F. B., was an obese fireman who continued to be obviously short of breath on effort although he denied symptoms; tilting him occasioned a

TABLE I

Showing all the data in this investigation

Lying horizontal basal										Tilted, passive 45°										Difference in positions												
Number	Age	Sex	Name	Weight kgm.	Height cm.	Body surface sq. meters	Blood volume liters	Basal metabolic			Arterio-venous			Cardiac output liters per min- ute	Pulse rate	Stroke volume cc.	Blood pressure	Vital capacity liters	Grollmann's		Basal metabolic rate cc. O ₂ per min- ute	Arterio-venous		Cardiac output liters per min- ute	Pulse rate	Stroke volume cc.	Blood pressure	Vital capacity liters	Cardiac output per cent	Pulse rate per cent	Stroke volume per cent	Blood pressure per cent
								cc. O ₂ per min- ute	cc. per liter	liters per min- ute	cc. per liter	cc. per min- ute	"Cardiac" index						"Cardiac" index													
1	30	F	A.C.	73	161	1.80	65	220	53	4.2	61	85	120/80	2.7	3.90	2.33	1.67	213	71	3.0	70	43	105/75	2.8	-28.6	+9.1	-33.9	-12.5				
2	38	F	H.B.	63	173	1.75	85	200	46	4.1	52	85	110/70	3.2	3.25	2.51	2.06	218	64	3.6	75	48	115/85	3.3	-18.2	+11.3	-13.5	+4.5				
3	38	F	M.H.	64	169	1.74	56	190	65	2.9	52	56	120/80	2.8	3.31	1.67	1.78	203	65	3.1	58	53	130/90	2.9	+0	+6.9	-5.4	+8.3				
4	52	F	L.D.	76	176	1.99	52	175	0	2.9	56	52	120/90	2.1	3.18	1.51	1.51	192	67	2.9	58	50	130/85	3.1	+0	+3.7	-3.8	+8.3				
5	51	F	G.S.	80	156	1.80	50	180	47	4.0	80	50	160/100	1.4	3.22	2.22	2.56	223	48	4.6	80	58	155/110	1.5	+15.0	+0	+16.0	-3.1				
6	57	M	P.C.	88	158	1.70	79	215	56	4.6	58	79	140/95	3.4	3.35	2.31	1.91	220	67	3.3	80	41	130/95	3.5	+21.2	+21.2	-16.0	-7.1				
7	48	M	H.D.	81	175	1.97	65	240	52	3.8	61	65	125/85		3.30	2.10	1.92	252		3.4	70	48	127/90		-5.0	+15	-14.0	-0.3				
			Average																													
			Varicose subjects before op.																													
1	41	F	E.C. ⊕*	75	163	1.81	90	214	40	6.1	68	90	125/80	2.8	3.05	3.37	2.70	256	52	4.9	88	56	140/90	2.7	-19.7	+29.4	-37.8	+12.0				
2	65	M	T.S. ⊕	66	161	1.70	5.2	261			68		185/100	2.2		2.65	277	62	4.5		60	75	150/100	2.4		0		-18.9				
3	40	F	S.D. ⊕*										130/80	2.7				Fainted		84				3.2		+23.5		->50				
4	53	M	B.A.	64	156	1.61	6.2	248	39	6.4	80	80	135/70	3.2	3.90	3.90	2.82	260	54	4.8	84	57	115/80	3.2	-25.0	+5.0	-28.7	-14.8				
5	36	M	F.K. ⊕	77	169	1.88	6.0	268	42	6.2	64	97	125/85	2.7	3.25	3.30	2.08	273	70	3.9	88	44	125/85	3.1	-37.1	+37.6	-54.6	0				
6	43	F	G.B. ⊕*	75	165	1.82	93	231	43	5.4	58	93	150/80	2.4	3.31	2.97	2.42	246	56	4.4	72	61	130/80	2.3	-18.5	+21.2	-31.4	-13.3				
7	50	F	M.MacD.*	80	168	1.90	6.3	315	54	5.8	64	91	170/105	2.5	3.31	3.05	2.26	340	80	4.3	82	52	150/105	2.7	-25.9	+28.1	-12.9	-11.8				
8	48	F	R.B.	77	158	1.79	5.7	218	51	4.3	68	63	125/75	2.1	3.18	3.02	2.14	229	71	4.1	84	49	120/80	2.5	-29.3	+17.7	-49.5	-4.0				
9	47	M	A.M.	93	156	1.92	6.3	262	45	5.8	60	97	150/100	2.5	3.28	3.02	2.65	280	70	4.1	84	49	130/90	2.7	+2.0	+33.3	-43.3	-4.2				
10	55	M	M.N. ⊕	70	180	1.89	5.9	236	48	4.9	74	66	120/80	3.4	3.18	2.59	2.65	237	47	5.0	98	51	115/90	3.6	+3.0	+42.4	-22.7	-4.2				
11	61	M	P.D. ⊕	71	173	1.84	5.5	217	47	4.6	58	79	150/90	4.3	3.29	2.50	2.34	234	55	4.3	80	54	120/90	4.4	-6.5	+38.0	-31.6	-20.0				
12	39	M	F.B.*	107	178	2.21	7.5	307	67	4.6	56	82	130/85	3.4	3.35	2.05	1.47	317	95	3.3	78	42	115/85	3.5	-28.3	+29.3	-48.8	-11.5				
			Average																													
			After op.																													
1		F	E.C.*	72	169	1.79	5.3	220	53	4.1	64	64	120/74	2.5	3.23	2.29	2.26	231	57	4.0	70	57	130/82	2.6	-1.2	+9.3	-10.9	+8.3				
4		M	B.A.	64	156	1.61	5.7	195	45	4.3	72	60	120/70	2.6	3.23	2.62	2.31	205	54	3.8	78	49	110/70	2.7	-11.8	+8.3	-18.3	-8.3				
11		M	P.D.	72	173	1.85	5.7	189	47	4.0	55	73	140/80	4.3	3.11	2.16	1.89	198	57	3.5	59	67	130/85	4.2	-12.5	+7.3	-18.3	-7.2				
6		F	G.B.*	80	165	1.87	7.1	218	48	5.2	57	91	140/80	2.4	3.11	2.78	2.08	260	60	3.9	62	74	140/90	2.5	-25.0	+8.8	-18.3	0				
7		F	M.MacD.*	82	168	1.92	7.1	272	41	6.2	64	97	145/105	2.4	3.24	3.23	2.92	286	49	5.6	82	68	125/90	2.5	-9.7	+28.1	-29.9	-13.8				
12		M	F.B.*	111	178	2.28	7.4	325	52	6.3	61	99	115/70	3.2	3.24	2.76	1.75	341	85	4.0	81	49	110/80	3.5	-36.5	+26.6	-50.6	-4.3				
5		M	F.K.	72	169	1.79		220			68		126/88	2.5		2.64	2.20				76	60	126/84	2.7	-16.1	+11.0	-22.7	-0.0				
			Average																													

* Patients with symptoms.

⊕ = electrocardiographs taken in both positions.

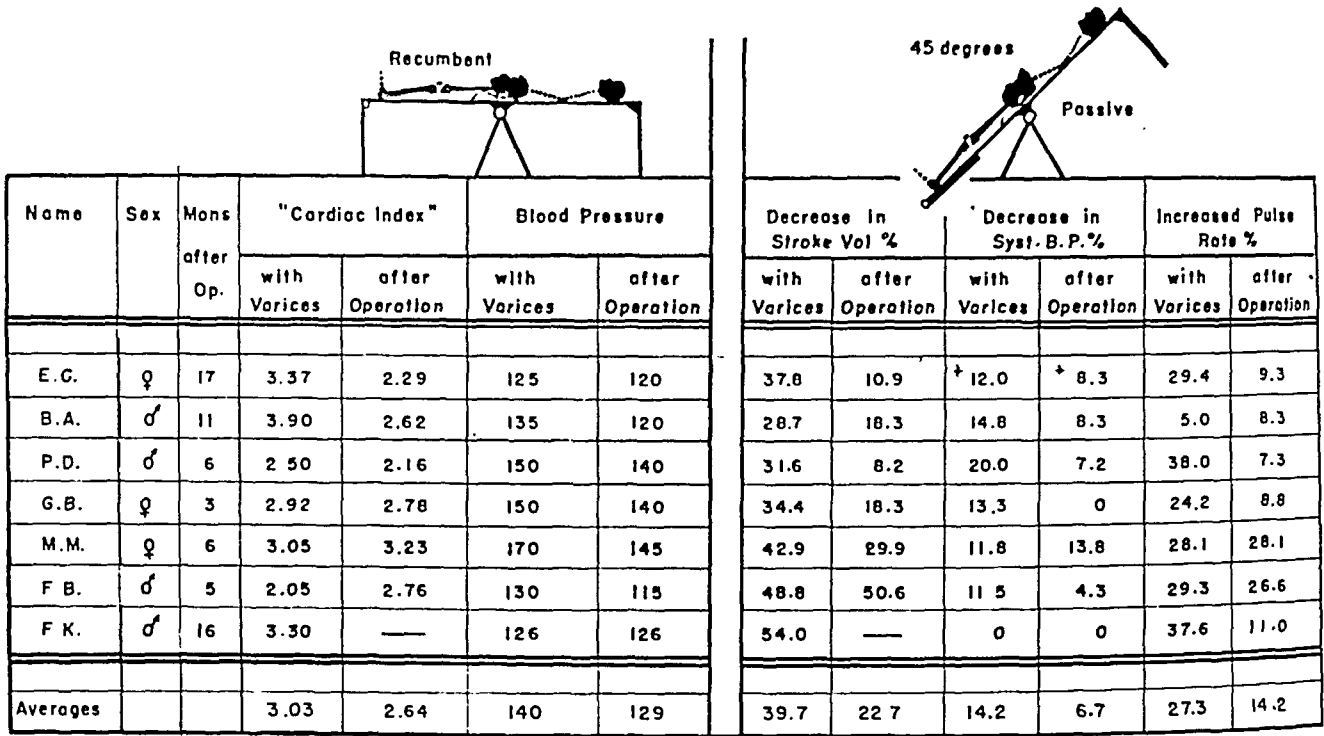


FIG. 3. CIRCULATORY FUNCTIONS BEFORE AND AFTER SAPHENOUS LIGATION FOR VARICOSE VEINS

great fall in stroke volume but this was compensated by a considerable increase in the pulse rate. The explanation for both these subjects may be that the obliteration of the varices is incomplete.

Table I contains all the data in our investigation and from this we have prepared Figures 2 and 3. Referring to Table I one notes that the changes in blood volume per square meter of body surface suggest that less blood is actually present in circulation after the venous reservoirs are removed, however, this comparative data is too limited for interpretation. The vital capacity of these subjects showed no consistent changes. Electrocardiograms were done in the recumbent and tilted positions on the 7 subjects indicated. There was a slight lowering of the T-wave in Lead II in the tilted positions of T. S. and F. K., a known physiologic change (9). However, in S. D. there was marked lowering of T₁ and T₂ and slurring of QRS₂ and fifteen minutes after the graph was taken she fainted and the blood pressure became unobtainable. This may have been a reaction to postural hypotension as an exaggerated response to her peripheral pooling. In a recent careful study of 3 patients with postural hypotension, Stead and Ebert (10) did not state that their subjects were without varicose veins.

Probably the most significant observation we made was the increase in pulse rate on tilting. Comparison of the two last columns in Figure 3 indicates this response. The definite rise in pulse rate on tilting is of course a compensatory mechanism to offset the fall in stroke volume and to maintain the cardiac output. The average increase in pulse rate of all 7 before ligating the veins is twice that after ligation. By application of the previous formula,³ this indicates to us that circulatory efficiency has been definitely increased by removal of the blood pool in the varicose veins.

CONCLUSIONS

We believe that undue fatigue, shortness of breath, dizziness, fainting, and precordial distress may be occasioned by the pooling of blood in varicose veins. These clinical investigations offer evidence that the circulatory efficiency is decreased by such extensive pooling and that removal of this peripheral blood reservoir restores the hemodynamics of the subjects toward normal and relieves their symptoms.

We are indebted to Dr. W. H. Forbes for doing the blood volume determinations and his advice.

³ See footnote 2.

PROTOCOLS

Case 1.⁴ E. C.: A 44-year old woman managing a farm. She had bilateral varicose veins for at least 12 years and complained of some undue shortness of breath, fatigue, and occasional dizzy spells while at work. Following ligation and injection she was greatly improved and noted less fatigue and shortness of breath.

Case 2. T. S.: A 65-year old gardener who said his varicose veins had been present for 35 years. They were particularly large on the left side and extended to the groin. He denied discomfort in any way.

Case 3. S. D.: A 40-year old Polish housewife who had bilateral varicose veins for 7 years. She spoke little English, but no history of dyspnea, dizziness, or fainting was obtained. However, on tilting her she had a fall in blood pressure and fainted, making further study impossible.

Case 4. B. A.: A 53-year old Hebrew storekeeper with large bilateral varices of 7 years duration. He worked standing each day and denied symptoms except some aching in the legs. However, he wore supportive bandages most of the time.

Case 5. F. K.: A 36-year old meat smoker (the man in the glass house at the World's Fair) who for 3 years had huge varicose veins reaching both knees. He denied symptoms but after operation he observed that he could now walk 5 to 10 miles without fatigue or shortness of breath.

Case 6. G. B.: A 43-year old housewife who for 19 years had large varicosities of both legs, more extensive on the right where they reached the groin. She complained of undue shortness of breath and often sighed heavily and was frequently dizzy. Following ligation and injection she was greatly improved and had a greater tolerance for work.

Case 7. M. MacD.: A 50-year old housewife who had large varicose veins coursing up both legs to the groin. For 20 years these had troubled her. She complained of shortness of breath and also of sudden attacks of precordial pain and dizziness when she got out of bed and stood up. On several occasions she had fallen in a faint. After ligation of her veins she was greatly improved and no longer had such attacks. However, moderate varices in the right leg persisted.

Case 8. R. B.: A 48-year old Polish housewife who had bilateral varicose veins of 10 years duration. She did not complain.

Case 9. A. M.: A 47-year old Russian émigré woman with large varicosities extending above both knees.

⁴We consider cases 1, 3, 6, 7, and 12 to have the symptoms described.

These had been present many years and she had no cardio-respiratory symptoms.

Case 10. M. N.: A 55-year old Irish workman who had had a large pattern of varicose veins extending above his knees for the previous 20 years. He was a tense, restless fellow smoking 40 cigarettes daily and denying symptoms referable to his circulatory system.

Case 11. P. D.: A 64-year old retired fireman with the varicosities shown in Figure 1. He was without particular complaint, but said he felt better and fatigued less after their obliteration.

Case 12. F. B.: A 39-year old obese railroad fireman who had bilateral varicosities for 20 years. They were larger on the right and reached above the knee. He denied symptoms but was obviously short of breath on slight effort.

BIBLIOGRAPHY

1. Gay, J., *On Varicose Disease of the Lower Extremities and Its Allied Disorders: Skin Discoloration, Induration and Ulcer; being the Lettsomian Lectures 1867.* John Churchill and Sons, London, 1868.
2. Lee, W. E., and Freeman, N. E., Circulatory disturbances produced by extensive angiomas of the lower extremities associated with varicose veins. *Ann. Surg.*, 1940, 112, 960.
3. Asmussen, E., Christensen, E. H., and Nielsen, M., The regulation of circulation in different postures. *Surgery*, 1940, 8, 604.
4. Grollman, A., *The Cardiac Output of Man in Health and Disease.* C. C. Thomas, Baltimore, 1932.
5. Sweeney, H. M., and Mayerson, H. S., Effect of posture on cardiac output. *Am. J. Physiol.*, 1937, 120, 329; Mayerson, H. S., Sweeney, H. M., and Toth, L. A., Influence of posture on circulation time. *Am. J. Physiol.*, 1939, 125, 481.
6. Schneider, E. C., and Crampton, C. B., The effect of posture on the minute volume of the heart. *Am. J. Physiol.*, 1934, 110, 14.
7. Gibson, J. G., 2nd, and Evans, W. A. Jr., Clinical studies of the blood volume. II. The relation of plasma and total blood volume to venous pressure, blood velocity rate, physical measurements, age, and sex in ninety normal humans. *J. Clin. Invest.*, 1937, 16, 317.
8. Kerr, W. J., The treatment of angina pectoris by methods which appear to promote more adequate filling of the heart. *Am. Heart J.*, 1938, 16, 544.
9. Scherf, D., and Weissberg, J., The alterations of the T-waves caused by a change of posture. *Am. J. M. Sc.*, 1941, 201, 693.
10. Stead, E. A., Jr., and Ebert, R. V., Postural hypotension. *Arch. Int. Med.*, 1941, 67, 546.

TISSUE THIAMIN CONCENTRATIONS AND URINARY THIAMIN EXCRETION¹

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INTRODUCTION

Abnormalities of thiamin excretion are observed chiefly in patients in whom the diagnosis of deficiency is fairly obvious on the clinical grounds of history and physical examination (1 to 4). The deviations from normal excretion in individuals suffering from mild or subclinical deficiency are usually equivocal. These observations suggest that the changes in tissue thiamin concentrations in human deficiency are not great or that the changes which occur have a relatively slight effect upon the urinary excretion of this vitamin. In the following study, the concentrations of thiamin in human tissue have been determined and the variations observed have been compared with the changes found in thiamin excretion in individuals with overt deficiency. Supplementary animal experiments have permitted direct observation of the relationship between tissue changes and changes in vitamin excretion.

METHODS

a. Tissue analyses

The thiochrome method has been used essentially as in earlier studies of the thiamin content of tissues (5). The small amount of thiamin in human tissue has required the extraction of somewhat larger quantities of tissue and has proportionally increased the error due to the inclusion of irrelevant fluorescent materials in the final thiochrome measurement.

Human tissues are finely divided in a Waring Blendor. Five gram aliquots are extracted with 50 milliliters of 0.1 normal sulfuric acid for 1 hour at 100 degrees centi-

grade with an occasional stirring. The pH is adjusted to 4.5 with sodium acetate and the extracts are cooled and centrifuged. The solid material is discarded and the supernatant is incubated overnight at 37 degrees centigrade with 1 gram of takadiastase to complete hydrolysis of the cocarboxylase. Five milliliter aliquots of the incubated extract are treated with alkaline ferricyanide, and the thiochrome extracted with 15 milliliters of isobutyl alcohol. The concentration of thiochrome in the butanol is estimated with a photoelectric fluorometer.

Calculations of the thiamin content of the tissues are based upon the recovery of thiamin obtained in duplicate aliquots of extract to which known amounts of thiamin have been added. The errors in the method are of the order of minus 15 per cent, due to incomplete extraction of thiamin from the tissue, and plus 30 per cent, due to inclusion of irrelevant fluorescent materials in the final thiochrome measurement. These errors may be decreased by repeating the extraction procedure and by using permuit columns to diminish the amount of irrelevant fluorescent material (6, 7).

b. Urine analyses

The thiochrome method has been used essentially as in earlier studies of thiamin excretion (7).

All patients and animals are fasted overnight before being tested.

Urine collections of short duration in animals are secured by tying the urethra under novocaine anesthesia at the beginning of the collection period. At the conclusion of the experiment the animal is killed by decapitation, the bladder is removed in toto, and the urine transferred to a small collecting vessel. Urine collections of greater duration than 4 hours are obtained by the use of small metabolism cages.

For tolerance tests in patients, a base line of excretion is established from a urine specimen taken immediately preceding the test. In animal experiments, the base line is estimated from the excretion observed in control animals, normal and deficient, that have been given saline or water instead of vitamin.

For the per oram test in man, 5 milligrams of thiamin hydrochloride are administered with the mid-day meal. The procedures for parenteral tests in man are indicated in Table V.

¹ Aided by a grant from the R. R. Williams and R. E. Waterman Fund for the Investigation of Nutritional Disease, Research Corporation, New York City.

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TABLE III

Concentration of thiamin in human tissue

Calculations expressed as micrograms thiamin per gram tissue.

Subject	Age in years	Sex	Heart	Skeletal muscle	Liver	Kidney cortex	Cerebral cortex	Remarks
Presumably normal individuals	0	Female	1.8	1.4	1.1			6 months fetus
	0	Female	1.8*	1.5*	0.9*	0.9*	0.5*	8 months fetus
	10	Male	3.5	1.2	1.4	2.1		Food in stomach, traumatic death
	30	Male	2.4	0.4	1.0	1.8	1.1	Negro, traumatic death
	52	Male	2.0	0.4	1.2	1.3	0.8	Traumatic death, survived 24 hours
Patients with good nutrition	0	Female		1.3*			0.3*	Omphalocele
	0	Female	3.5	2.7	1.5	2.8		Meningoencephalocele
	7 weeks	Male			1.3		0.6*	Pneumonia 2 weeks
	10 months	Female	3.3	1.6	1.1	3.1		Fulminating sepsis
	5 years	Male	3.2	1.0	1.1	1.4		Leukemia 6 months
	6	Female	3.8		1.9	1.9	1.0	Neuroblastoma of adrenal
	37	Male		0.4	1.0	1.0		Brain tumor
	48	Male	2.4	0.5	1.4	1.3		Cerebral metastases
	51	Male	2.3	0.4	1.1	1.7	1.0	Brain tumor
	58	Male	1.7	0.4	1.1	1.0	1.1	Brain tumor
	61	Female	1.9	0.4	1.0	1.3	1.0	Brain tumor
Patients with fair nutrition	26	Male	1.3	0.4	0.7	1.2		Acute rheumatic carditis
	47	Female	1.4	0.2	0.7	0.7		Heart failure with anasarca
	50	Male	1.9	0.5	1.1	1.2	1.0	Alcoholic coma, cerebral injury
	54	Male	1.3	0.2	1.0	1.2		Cirrhosis, gastric hemorrhage
	68	Male	1.4	0.4	1.0	1.0		Calcified aortic valve, cardiac failure
Patients with poor nutrition	34	Female	0.9	0.2	0.7	1.0		High cord injury, sepsis 3½ months
	37	Male	0.5*	0.1*	0.5*	0.5*	0.6*	Alcoholism, tuberculosis
	38	Female	0.6	0.0	0.3	0.4	0.5	Active tuberculosis of spine and adrenals; high fever
	49	Female	0.5	0.1	0.6	0.3		Lymphosarcoma, uremia; high fever for 3 weeks

* Permutit column analyses.

The thiamin concentrations observed in adult patients, after the administration of large quantities of thiamin, are presented in Table IV. The concentration in some tissues appears higher than the usual normal.

b. Thiamin excretion in patients

The tendency of thiamin deficient patients to excrete less thiamin than normal subjects is illustrated in Table V. Since the observations were made on patients with different degrees of deficiency, the sensitivities of the various tests cannot be compared.

The effect of a restricted intake of thiamin, 300 micrograms per day, was studied in an ambulatory psychiatric patient in good physical and fair men-

tal condition. The daily excretion of thiamin fell promptly from an initial level of 60 micrograms to a level of 5 to 10 micrograms. The excretion of parenterally administered vitamin changed but little. At the beginning of the experiment, 5 per cent of a subcutaneous tolerance test of 200 micrograms was excreted in 4 hours. Two days later 11 per cent of a subcutaneous test of 600 micrograms was excreted in 4 hours. After 2 weeks of deficient diet the tests were repeated. Four per cent of the 200 microgram test was excreted and 10 per cent of the 600 microgram test. The excretion of orally administered thiamin, on the other hand, had become frankly abnormal. Less than 10 per cent of a 5 milligram test was excreted in a 24 hour period (2). With the ad-

For the *per oram* test in animals, 160 micrograms of thiamin and 160 micrograms of riboflavin are administered by stomach tube in 4 milliliters of water. Urine is collected for 24 hours, the animals fasting, with water *ad lib*. For the subcutaneous test, 40 micrograms of thiamin and 40 micrograms of riboflavin are injected subcutaneously in the right axilla in 1 milliliter of normal saline solution. Urine is collected for 3½ hours, the animals fasting, with water *ad lib*. For the intravenous tolerance test, 40 micrograms of thiamin and 40 micrograms of riboflavin are injected intravenously in 1 milliliter of normal saline solution. Four milliliters of water are administered by stomach tube and urine is collected for 1½ hours. Control experiments indicate that these periods are sufficient to permit the excretion of thiamin to return to the basal level. Measurement of riboflavin excretion (8) serves in the tests as a control for variables of absorption and renal function.

c. Diets

The deficient diet used in the study of the psychiatric patient was the basal diet without supplementary vitamins, described by Williams *et al.* (9).

The control diet for the animal experiments was that described by Peters and Rossiter (10), except that autolyzed yeast (Vegex) was substituted for their dried yeast. The deficient diet was obtained by autoclaving the autolyzed yeast for 2 hours at 20 pounds pressure before mixing it with the other ingredients.

RESULTS

a. Thiamin content of human tissues

A comparative study with the yeast fermentation method (11), in which the sources of error are different from those in the thiochrome technique, has verified the approximate validity of the thiamin analyses (Table I).

A study of the effect of post-mortem autolysis upon the thiamin content of tissues has failed to show significant change, plus or minus 10 per cent, following 24 hours' incubation at 37 degrees centigrade, or 48 hours' preservation at 0 degrees centigrade. Corroborative evidence for the validity of post-mortem analyses is obtained from a comparison of autopsy and biopsy analyses of brain and muscle of human subjects (Table II).

The range of thiamin concentrations found in human tissues is illustrated in Table III. The patients have been grouped on the basis of their probable nutritional status as judged by clinical circumstances and dietary histories. In general the concentrations of thiamin found in the tissues are in agreement with the grouping. Tissues

TABLE I

Comparison of yeast fermentation method and thiochrome method of determining the thiamin content of human tissue

Calculations expressed as micrograms thiamin per gram fresh tissue.

Heart		Brain		Liver		Kidney		Skeletal muscle	
T*	Y†	T	Y	T	Y	T	Y	T	Y
1.4	2.2			1.4	1.3				
				1.0	0.9	1.0	1.1	0.4	0.3
				1.2	0.8			0.5	0.4
1.9	3.1	1.0	1.2	1.0	1.1	1.3	1.5	0.4	0.5
2.2	3.0								
1.9	3.0								
1.9	1.9								
3.5	3.6								
2.0	2.5	1.2	1.3						
3.5	2.8							1.8	1.2
2.3	2.3								
4.9†	4.9†								
2.0	1.8								
1.2	1.1								
1.1	1.1								
1.7	2.0								
2.7	3.6			2.4	2.0				
4.1	4.2								
1.4	1.0								

* T = Thiochrome method.

† Y = Yeast fermentation method.

† 5.0 by rat curative assay, done through the courtesy of Dr. W. L. Sampson, Merck Institute for Therapeutic Research, Merck and Company, Rahway, New Jersey.

TABLE II

Comparison of concentrations of thiamin found in human tissues at operation and autopsy

Tissue	Source	Thiamin per gram tissue
		micrograms
Brain	Autopsy	1.1
Brain	Biopsy	1.2
Skeletal muscle	Autopsy	0.6
Skeletal muscle	Autopsy	0.4
Skeletal muscle	Biopsy	0.5
Skeletal muscle	Biopsy	0.4

from young individuals appear to contain more thiamin per gram than do tissues from older persons. A possible exception is cerebral cortical tissue which in immature infants contains very little thiamin (12).

Symptoms of thiamin deficiency were recognized in only one patient in Table III. In the group with poor nutrition, the alcoholic with tuberculosis complained of pain and paresthesia in the lower extremities during the period when he was still ambulatory. The other patients were not ambulatory at a time when exercise might have produced symptoms of deficiency.

TABLE III
Concentration of thiamin in human tissue

Calculations expressed as micrograms thiamin per gram tissue.

Subject	Age in years	Sex	Heart	Skeletal muscle	Liver	Kidney cortex	Cerebral cortex	Remarks
Presumably normal individuals	0	Female	1.8	1.4	1.1			6 months fetus
	0	Female	1.8*	1.5*	0.9*		0.5*	8 months fetus
	10	Male	3.5	1.2	1.4	0.9*	2.1	Food in stomach, traumatic death
	30	Male	2.4	0.4	1.0	1.8	1.1	Negro, traumatic death
	52	Male	2.0	0.4	1.2	1.3	0.8	Traumatic death, survived 24 hours
Patients with good nutrition	0	Female		1.3*			0.3*	Omphalocele
	0	Female	3.5	2.7	1.5	2.8		Meningoencephalocele
	7 weeks	Male			1.3		0.6*	Pneumonia 2 weeks
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	5 years	Male	3.2	1.0	1.1	1.4		Leukemia 6 months
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	37	Male		0.4	1.0	1.0		Brain tumor
	48	Male	2.4	0.5	1.4	1.3		Cerebral metastases
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	61	Female	1.9	0.4	1.0	1.3	1.0	Brain tumor
Patients with fair nutrition	26	Male	1.3	0.4	0.7	1.2		Acute rheumatic carditis
	47	Female	1.4	0.2	0.7	0.7		Heart failure with anasarca
	50	Male	1.9	0.5	1.1	1.2	1.0	Alcoholic coma, cerebral injury
	54	Male	1.3	0.2	1.0	1.2		Cirrhosis, gastric hemorrhage
	68	Male	1.4	0.4	1.0	1.0		Calcified aortic valve, cardiac failure
Patients with poor nutrition	34	Female	0.9	0.2	0.7	1.0		High cord injury, sepsis 3½ months
	37	Male	0.5*	0.1*	0.5*	0.5*	0.6*	Alcoholism, tuberculosis
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* Permutit column analyses.

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The tendency of thiamin deficient patients to excrete less thiamin than normal subjects is illustrated in Table V. Since the observations were made on patients with different degrees of deficiency, the sensitivities of the various tests cannot be compared.

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tal condition. The daily excretion of thiamin fell promptly from an initial level of 60 micrograms to a level of 5 to 10 micrograms. The excretion of parenterally administered vitamin changed but little. At the beginning of the experiment, 5 per cent of a subcutaneous tolerance test of 200 micrograms was excreted in 4 hours. Two days later 11 per cent of a subcutaneous test of 600 micrograms was excreted in 4 hours. After 2 weeks of deficient diet the tests were repeated. Four per cent of the 200 microgram test was excreted and 10 per cent of the 600 microgram test. The excretion of orally administered thiamin, on the other hand, had become frankly abnormal. Less than 10 per cent of a 5 milligram test was excreted in a 24 hour period (2). With the ad-

TABLE IV

Concentration of thiamin in tissues of patients receiving thiamin therapy

Calculations expressed as micrograms thiamin per gram fresh tissue.

Age in years	Sex	Heart	Skeletal muscle	Liver	Kidney cortex	Cerebral cortex	Remarks
59	Male	2.6	0.8	1.3	2.1		Carcinomatosis. 10 mgm. thiamin intravenously, each day <i>ad</i> 25
76	Female	1.3	0.7	1.2	1.2		Carcinoma of mouth. 3 mgm. thiamin by mouth for 3 weeks, none for 2 weeks
75	Male	2.3					Carcinoma of stomach; cachexia. B complex intramuscularly for 10 days
40	Female					1.2	Cerebral tumor. 10 mgm. B ₁ by mouth, each day, for 1 week
18	Female	4.9					Encephalitis? 50 mgm. thiamin each day, 3 weeks; 100 mgm. nicotinic acid each day, for 1 week, up to day of death
38	Male	4.3		2.4			Cirrhosis. 30 grams Brewer's yeast each day, 30 days; 20 mgm. B ₁ intramuscularly or subcutaneously each day, 21 days; 5 cc. liver extract intramuscularly weekly up to 2 days before death

TABLE V

A comparison of the amounts of thiamin excreted by normal individuals and by individuals whose diets have been deficient in thiamin

Micrograms thiamin excreted		Type of test
Normal subjects	Deficient subjects	
150 109 283	44 20 0	24 hour excretion.
252 280 230	15 50	24 hour excretion following subcutaneous injection of 0.5 mgm. B ₁ to normals and 1.0 mgm. B ₁ to deficient.
206 136	78 52	3 hour excretion following intramuscular injection of 1 mgm. B ₁ and B ₂ .
315 300 350	250 250 150	1 hour excretion following intravenous injection of 1.2 mgm. B ₁ and B ₂ .
Per cent of tolerance test excreted		Type of test
Normal subjects	Deficient subjects	
22 26 25 22 21	9 6 12	1 hour excretion following intravenous injection of 0.02 mgm. B ₁ and B ₂ per kgm. body weight.

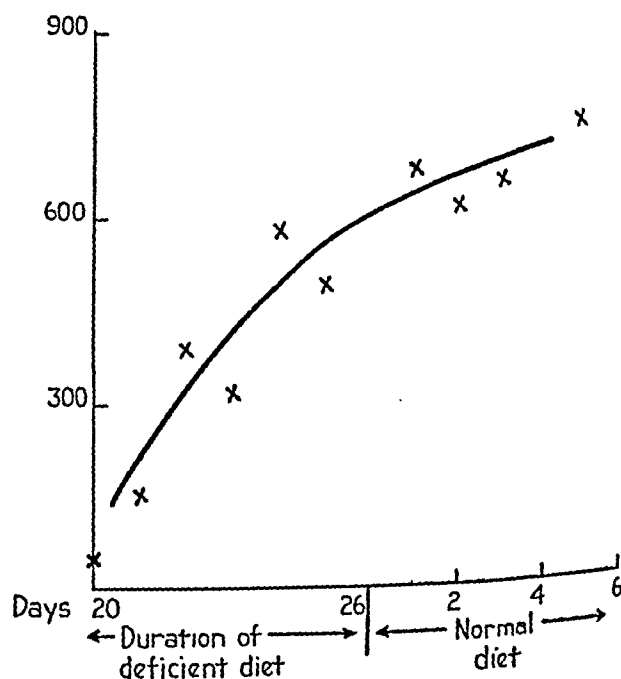
Micrograms B₁
excreted in urine

FIG. 1. AMOUNT OF THIAMIN EXCRETED IN THE DAILY URINE BY A DEFICIENT SUBJECT IN RESPONSE TO SUCCESSIVE TOLERANCE TESTS CONSISTING OF 5 MGm. OF THIAMIN HYDROCHLORIDE ADMINISTERED WITH EACH MIDDAY MEAL

The lower limit of normal for this type of test is an excretion of about 500 micrograms (2).

ministration of repeated 5 milligram tests, the excretion of orally administered thiamin gradually returned to normal (Figure 1).

c. Thiamin content of rat tissues

The effect of a thiamin deficient diet upon the

Micrograms B₁
per gram tissue

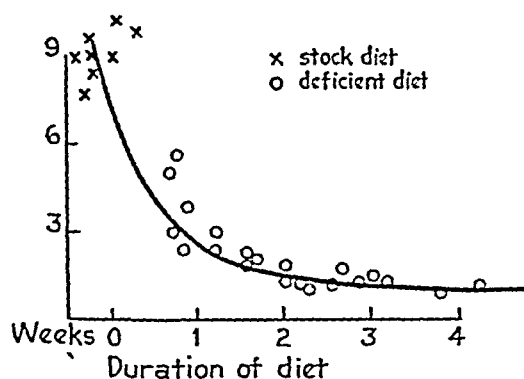


FIG. 2. EFFECT OF THIAMIN DEFICIENT DIET UPON CONCENTRATION OF THIAMIN IN LIVER OF RATS

Micrograms B₁
per gram tissue

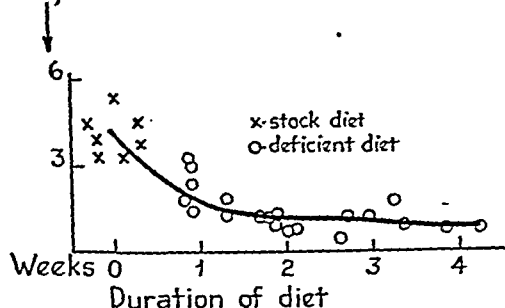


FIG. 3. EFFECT OF THIAMIN DEFICIENT DIET UPON CONCENTRATION OF THIAMIN IN KIDNEY OF RATS

thiamin content of the livers and kidneys of 180 gram male Wistar rats is illustrated in Figures 2 and 3. Muscle concentrations also fell from 1 microgram per gram to 0.5 microgram after 2 weeks of deficient diet. Symptoms of thiamin deficiency were not observed until the third week.

The effect of thiamin administration is illustrated in Figures 4, 5, and 6. In normal animals little increase in thiamin concentrations occurs (Figures 4 and 5). In deficient animals, the thiamin concentrations promptly return to normal (Figure 6).

d. Thiamin excretion in rats

In Figures 7 and 8 and in Table VI, the excretion of thiamin by normal animals is compared with the excretion of thiamin by thiamin deficient animals following tolerance tests of various types.

Micrograms B₁
per gram tissue

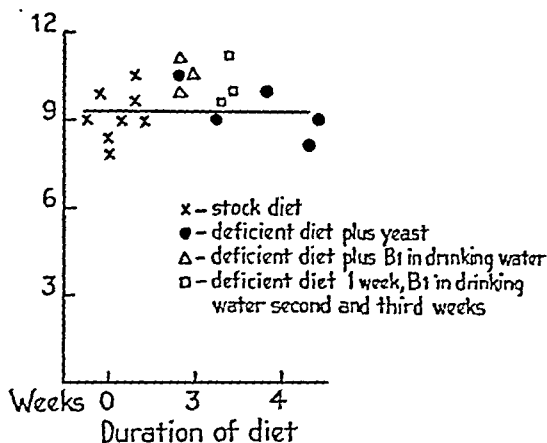


FIG. 4. EFFECT OF HIGH THIAMIN DIET UPON CONCENTRATION OF THIAMIN IN LIVER OF RATS

Micrograms B₁
per gram tissue

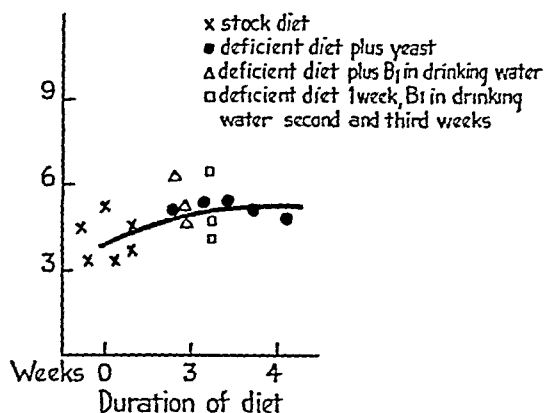


FIG. 5. EFFECT OF HIGH THIAMIN DIET UPON CONCENTRATION OF THIAMIN IN KIDNEY OF RATS

Differentiation of the normal animals from the deficient animals by means of these tests is generally possible. The chief confusion occurs when the intravenous test is applied to severely deficient animals that have lost 50 per cent of their body weight (Figure 7).

DISCUSSION

Our estimations of the concentrations of thiamin in rat tissues and their variations with diet are in agreement with observations made by other investigators (10, 13, 14). In the case of human

TABLE IV

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Calculations expressed as micrograms thiamin per gram fresh tissue.

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18	Female	4.9					Encephalitis? 50 mgm. thiamin each day, 3 weeks; 100 mgm. nicotinic acid each day, for 1 week, up to day of death
38	Male	4.3		2.4			Cirrhosis. 30 grams Brewer's yeast each day, 30 days; 20 mgm. B ₁ intramuscularly or subcutaneously each day, 21 days; 5 cc. liver extract intramuscularly weekly up to 2 days before death

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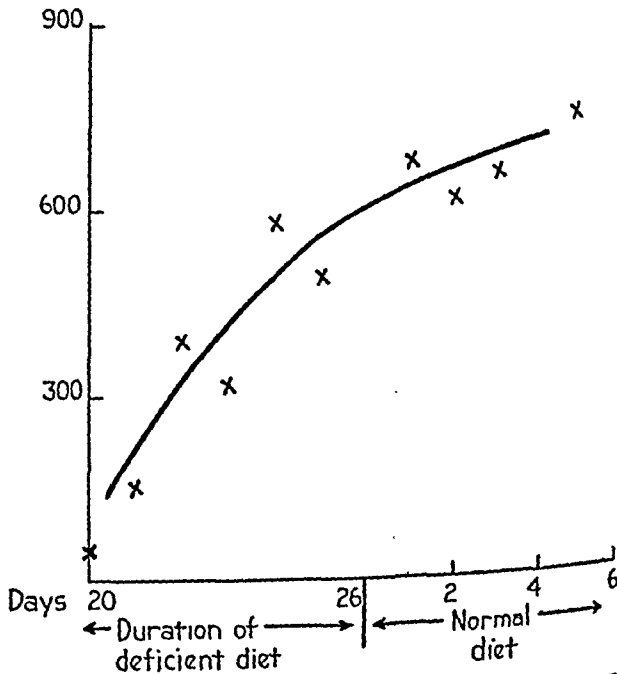


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Micrograms B₁
per gram tissue

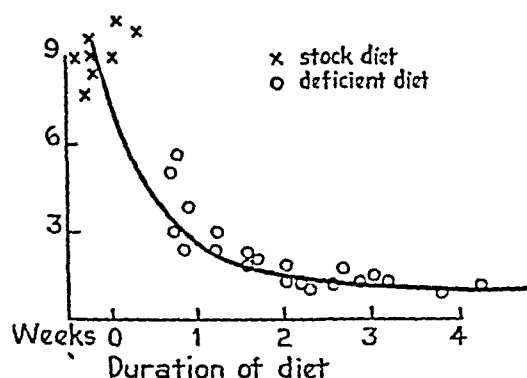


FIG. 2. EFFECT OF THIAMIN DEFICIENT DIET UPON CONCENTRATION OF THIAMIN IN LIVER OF RATS

Micrograms B₁
per gram tissue

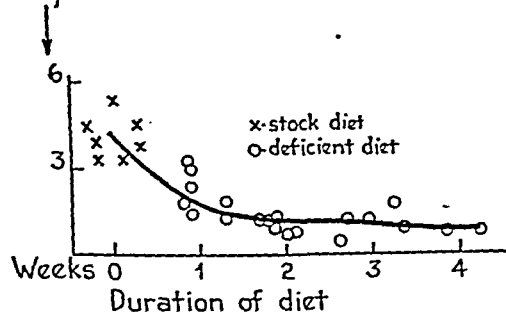


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Micrograms B₁
per gram tissue

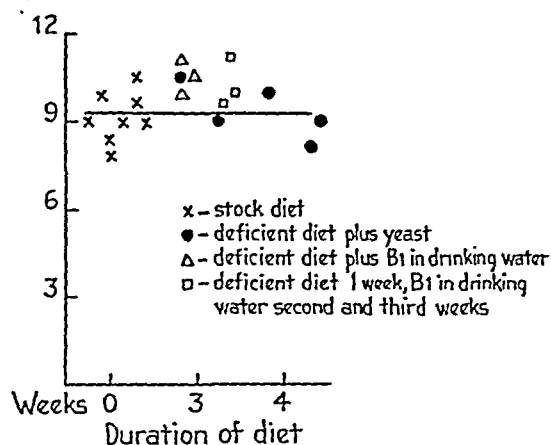


FIG. 4. EFFECT OF HIGH THIAMIN DIET UPON CONCENTRATION OF THIAMIN IN LIVER OF RATS

Micrograms B₁
per gram tissue

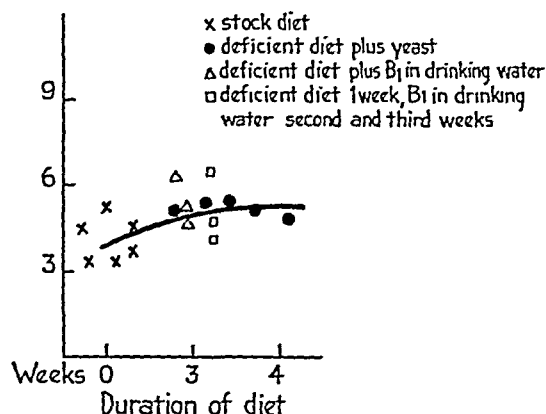


FIG. 5. EFFECT OF HIGH THIAMIN DIET UPON CONCENTRATION OF THIAMIN IN KIDNEY OF RATS

Differentiation of the normal animals from the deficient animals by means of these tests is generally possible. The chief confusion occurs when the intravenous test is applied to severely deficient animals that have lost 50 per cent of their body weight (Figure 7).

DISCUSSION

Our estimations of the concentrations of thiamin in rat tissues and their variations with diet are in agreement with observations made by other investigators (10, 13, 14). In the case of human

Micrograms B₁
per gram tissue

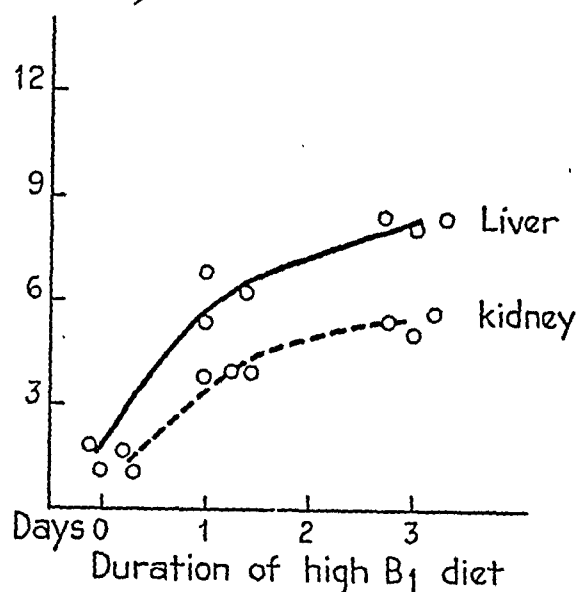


FIG. 6. EFFECT OF HIGH THIAMIN DIET UPON THE CONCENTRATION OF THIAMIN IN LIVER AND KIDNEYS OF RATS THAT HAD BEEN ON A DEFICIENT DIET FOR 2 WEEKS

Percent of injected
B₁ excreted in 1½ hours

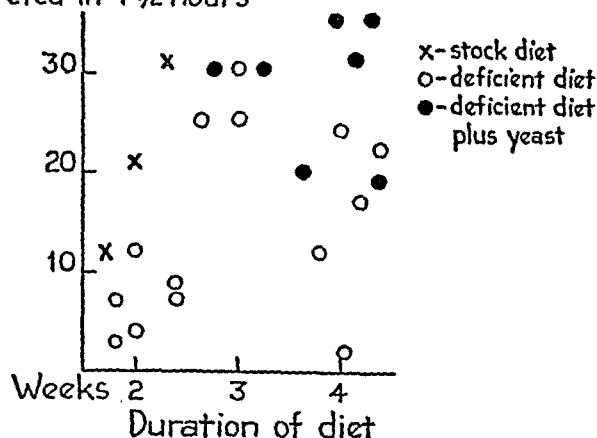


FIG. 7. EXCRETION OF THIAMIN BY NORMAL AND DEFICIENT RATS FOLLOWING THE INTRAVENOUS INJECTION OF 40 MICROGRAMS OF B₁ AND B₂

tissue, values with which our results might be compared have not appeared. The concentrations which we have found in man are of the order of 2 or 3 micrograms of thiamin per gram of heart muscle, 0.5 microgram per gram of skeletal muscle, and 1 microgram per gram of liver, kidney, and brain; the total for the average person being about 25 milligrams of thiamin. Concentrations significantly below these may be found in indi-

Percent of injected B₁
excreted in 3½ hours

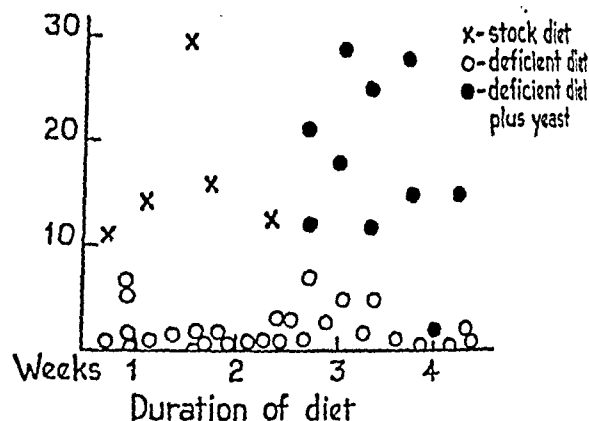


FIG. 8. EXCRETION OF THIAMIN BY NORMAL AND DEFICIENT RATS FOLLOWING THE SUBCUTANEOUS INJECTION OF 40 MICROGRAMS OF B₁ AND B₂

TABLE VI

A comparison of the micrograms of thiamin excreted in the urine during several tolerance tests performed on normal rats and on rats maintained for 2 weeks on a thiamin deficient diet

	Normal animals	Deficient animals	Normal controls*	Deficient controls*
Per os test. 24 hours	9.9	1.7	4.3	1.0
	18.6	0.8	3.1	1.3
	10.8	8.0	3.1	0.8
	6.0	2.0	2.4	1.3
Subcutaneous test. 3½ hours	6.9	0.5	0.6	0.3
	15.9	0.4	0.6	0.3
	6.0	0.5	0.7	0.3
		0.5	0.9	0.3
Intravenous test. 1½ hours	5.0	1.7	0.3	0.1
	8.6	1.5	0.6	0.2
	12.6	3.0	0.3	0.2
		4.8		

* Received water or saline instead of thiamin solution.

viduals suffering prolonged febrile illnesses, with dietary restrictions of an order sufficient to cause symptoms in active patients. Conversely, concentrations somewhat higher than the usual adult normal may be found in children and in patients who have recently received large quantities of the vitamin. In general, however, as with animals (15, 5), the ability to store added thiamin appears limited to short periods.

The tissue concentrations at which symptoms of deficiency develop are probably different in different tissues and probably vary with the amount of physiologic activity demanded of the tissue at the time of its deficiency. It is therefore not

surprising that in bedridden patients and in caged animals a considerable loss of thiamin may occur before symptoms definitely indicative of deficiency appear. Fortunately for the clinician, the amount of dietary restriction necessary to produce overt deficiency is usually sufficient to attract attention and to suggest the advisability of supplementary thiamin therapy. The occurrence of minor variations of thiamin concentrations in ambulatory patients, on the other hand, the so-called sub-clinical deficiencies, presents a complicated problem. Discussion of this type of deficiency will not be critical until biopsy and other studies permit an accurate appraisal of the interrelationships of dietary intake, tissue concentrations, and work performance.

The correlation between changes in tissue thiamin concentrations and changes in urinary thiamin excretion observed in our experiments appears definite but not particularly acute. In conformity with previous experiences (1 to 4), the excretion of individuals with clinically recognized deficiency was found sufficiently abnormal to permit their differentiation from normal subjects. In the single study of experimental deficiency in man (Figure 1) the *per oram* tolerance test appeared more sensitive than the parenteral test in detecting a minor degree of deficiency. However, a number of factors interfere with the general application of *per oram* tests (16, 17).

The quantitative relationship of the tissue changes and the excretory changes in our patients is difficult to evaluate without biopsy analyses. The tissue concentrations associated with frankly abnormal thiamin excretion might be estimated on clinical grounds to be of the order of the concentrations found in the autopsies of patients with poor nutrition (Table III). Past experience with measurements of thiamin excretion in patients with fair nutrition, such as those listed as "fair nutrition" in Table III, would not lead us to anticipate marked excretory changes in the latter group.

It has been a general experience that as the clinical evidence for thiamin deficiency becomes less secure, the differentiation afforded by the excretion test becomes proportionately less certain (1 to 4). The underlying difficulty appears to be that thiamin excretion is not a simple threshold phe-

nomenon. The dependence of rate and amount of excretion upon the size of the test dose and the route of administration (16), clearly indicates that the quantity excreted is not determined solely by the patient's nutritional status. The failure of normal subjects to excrete more than 20 to 40 per cent of the test vitamin is probably associated with a marked though temporary increase in the concentration of thiamin in their tissues (15, 5). The ability of tissue, both normal and deficient, to phosphorylate and hold thiamin for a few hours until it can be destroyed, considerably diminishes the working margin of tolerance tests. In the rat experiments, for example, striking changes in tissue concentrations were associated with differences in excretion that amounted to but a small percentage of the thiamin administered. In sick patients, evaluation of similar slight changes is complicated by the knowledge that physiologic variations, of the type exhibited in passing from the fasting to the absorptive state, may affect thiamin excretion as much as deficiency itself (16, 17). It is evident, therefore, that while excretion may be correlated with tissue concentrations under standard conditions (18), it is also unfortunately dependent upon renal function (3) and upon a number of variables which affect the rate at which thiamin is absorbed and distributed to the tissues, and the rate at which the tissues in turn can phosphorylate the vitamin, bind it to their protein (19), or destroy it. Control of these variables constitutes a major difficulty in the clinical application of tolerance tests.

SUMMARY AND CONCLUSIONS

1. The concentrations of thiamin in human tissue are of the order of 2 to 3 micrograms per gram for heart muscle, 0.5 microgram per gram for skeletal muscle, and 1 microgram per gram for brain, liver and kidney.
2. These concentrations may be temporarily increased by thiamin therapy, or they may be considerably reduced by inadequate diets.
3. Under comparable circumstances, deficient subjects tend to excrete less thiamin than normal subjects.
4. This tendency is not sufficient to permit recognition of small changes in tissue thiamin concentrations by measurements of thiamin excretion.

The writers wish to acknowledge their indebtedness to a number of individuals who helped materially in the completion of this work. The analyses by the Yeast Fermentation Method (Table I) were carried out at the Fleischmann Laboratories, Standard Brands, Inc., New York City, through the kindness of Dr. C. N. Frey, Dr. A. S. Schultz, and Dr. L. Atkin. The dietary study (Figure 1) was carried out at the New York Psychiatric Institute through the kindness of Dr. M. M. Harris. The biopsies of human brain and the studies of the effect of thiamin therapy upon tissue thiamin concentrations were obtained at the Neurological Institute, New York City, through the cooperation of Dr. A. Stowell. Material assistance in carrying out animal experiments and in making routine analyses was obtained from Dr. Norman Molomut, Mrs. Lillian Stout, Miss W. Greenspan, and Miss M. H. Carleen. Supplies of thiamin, riboflavin, cocarboxylase, and thiochrome were generously furnished by Merck & Co., Rahway, N. J.

BIBLIOGRAPHY

1. Westenbrink, H. G. K., and Goudsmit, J., Investigations on the relation between intake and excretion of aneurin in the case of normal subjects and pregnant women. *Arch. Néerl. de physiol.*, 1938, 23, 79.
2. Robinson, W. D., Melnick, D., and Field, H., Jr., Urinary excretion of thiamin in clinical cases and the value of such analyses in the diagnosis of thiamin deficiency. *J. Clin. Invest.*, 1940, 19, 399.
3. Najjar, V. A., and Holt, L. E., Jr., Studies in thiamin excretion. *Bull. Johns Hopkins Hosp.*, 1940, 67, 107.
4. Carden, G. A., Province, W. D., and Ferrebee, J. W., Clinical experiences with the measurement of the urinary excretion of vitamin B₁. *Proc. Soc. Exper. Biol. and Med.*, 1940, 45, 1.
5. Ferrebee, J. W., The effect of adrenalectomy on the phosphorylation of vitamins B₁ and B₂. *J. Biol. Chem.*, 1940, 136, 719.
6. Hennessy, D. J., and Cerecedo, L. R., The determination of free and phosphorylated thiamin by a modified thiochrome assay. *J. Am. Chem. Soc.*, 1939, 61, 179.
7. Ferrebee, J. W., and Carden, G. A., A procedure for the routine determination of vitamin B₁ in urine. *J. Lab. and Clin. Med.*, 1940, 25, 1320.
8. Ferrebee, J. W., The urinary excretion of riboflavin: fluorometric methods for its estimation. *J. Clin. Invest.*, 1940, 19, 251.
9. Williams, R. D., Mason, H. L., Wilder, R. M., and Smith, B. F., Observations on induced thiamine (vitamin B₁) deficiency in man. *Arch. Int. Med.*, 1940, 66, 785.
10. Peters, R. A., and Rossiter, R. J., Thyroid and vitamin B₁. *Biochem. J.*, 1939, 33, 1140.
11. Schultz, A. S., Atkin, L., Frey, C. N., and Williams, R. R., Application of the sulfite cleavage of thiamin to the yeast fermentation method. *J. Am. Chem. Soc.*, 1941, 63, 632.
12. Ferrebee, J. W., Weissman, N., Parker, D., and Owen, P. S., The thiamin content of human tissue. *Assoc. for Research in Nervous and Mental Disease*. 1941. In press.
13. Schultz, A. S., Light, R. F., Cracas, L. J., and Atkin, L., The concentration of vitamin B₁ in the tissues of the rat. *J. Nutrition*, 1939, 17, 143.
14. Williams, R. J., Studies on the vitamin content of tissues. *Univ. of Texas Publication*, No. 4137, 1941.
15. Westenbrink, H. G. K., and Goudsmit, J., Investigations on the aneurin- and cocarboxylase content of animal tissues, estimated by the thiochrome method. *Enzymologia*, 1938, 5, 307.
16. Melnick, D., Field, H., Jr., and Robinson, W. D., A quantitative chemical study of the urinary excretion of thiamine by normal individuals. *J. Nutrition*, 1939, 18, 593.
17. Melnick, D., Robinson, W. D., and Field, H., Jr., Fate of thiamine in the digestive secretions. *J. Biol. Chem.*, 1941, 138, 49.
18. Mason, H. L., and Williams, R. D., The urinary excretion of thiamin as an index of the nutritional level: assessment of the value of a test dose. *J. Clin. Invest.*, 1942, 21, 247.
19. Green, D. E., Westerfeld, W. W., Vennesland, B., and Knox, W. E., Pyruvic and α -ketoglutaric carboxylases of animal tissues. *J. Biol. Chem.*, 1941, 140, 683.

THE EFFECT OF EXERCISE AND OF FOUR COMMONLY USED DRUGS ON THE NORMAL HUMAN ELECTROCARDIOGRAM, WITH PARTICULAR REFERENCE TO T WAVE CHANGES

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It is not yet sufficiently appreciated that there are many factors, aside from heart disease, which may affect the electrocardiogram. This is a matter of considerable importance for anyone making electrocardiographic interpretations. It becomes especially significant in those instances where the electrocardiogram is on the borderline of normality, or in attempting to evaluate the successive changes in electrocardiograms taken serially.

It is the T waves of the electrocardiogram which are most commonly and significantly affected by these extracardiac factors. A good example is digitalis, which may so alter the contour of the S-T segments and T waves as to make interpretation difficult, unless it is known or suspected that the drug has been given. Significant T wave changes may also result from the inhalation of tobacco smoke (1), alkalosis (2), and change in body position (3), merely to mention a few factors.

Recently we have taken a renewed interest in the effects of exercise and of certain commonly used drugs on the normal human electrocardiogram. Many of the studies in this connection have been carried out on animals (4 to 8), and few or none of the results are directly applicable to man, except as noted above. Furthermore, the results of the studies on man show no general agreement, and this has resulted in some confusion. Our plan of study has been simple and eminently practical, being designed more to demonstrate what changes occur than to show why.

METHODS

The effects of exercise, adrenaline, ergotamine tartrate, acetyl- β -methylcholine (mecholy), and atropine sulfate were studied with reference to electrocardiographic changes in five normal subjects. Right carotid sinus pressure was studied in four subjects. The three classical leads were used. The subjects were all healthy males without heart disease. Their ages were 21, 29, 30, 31, and

38 years. All had normal electrocardiograms. Three were physicians and the other two were familiar with controlled laboratory procedures.

Each experiment was preceded by a period of rest until the pulse and blood pressure were stabilized. All the electrocardiograms were taken with the subject in the sitting position. Sufficient time was allowed between experiments for the effect of the previous procedure completely to disappear. Exercise and mecholy were followed by three hours of rest before the next experiment was done. All the other tests were carried out on different days. A control tracing was taken after the subject had rested and immediately prior to starting each procedure. Repeated tracings were taken during the experiments at appropriate times (in order to obtain the maximum effect). Blood pressure, pulse, and respiratory rate, and general reaction were noted with each tracing, as well as the electrical axis of the QRS complexes.

INDIVIDUAL PROCEDURES

1. Exercise

A. Method. The electrocardiograms were taken before, during, and immediately after exercise, the subject sitting on an orthopedic exerciser, pumping, as on a bicycle, with the right leg. The tracings were taken after three and twelve minutes, while vigorous pumping was continued. Also, continuous tracings of Lead 2 were taken in three subjects, beginning just prior to stopping exercise and continuing for two minutes after all activity had ceased.

B. Results. Exercise lowered the T waves of Lead 2 in all electrocardiograms of the five subjects (Figure 1), though but slightly as a rule. The T wave in Lead 1 was lowered in all but one subject; that particular T wave showed no change. The T wave in Lead 3 was lowered in two subjects and was elevated in one subject (Table I).

C. Comment. Our finding that exercise lowers the T waves is contrary to current ideas generally held, based on records obtained after stopping exercise which are very different from those made

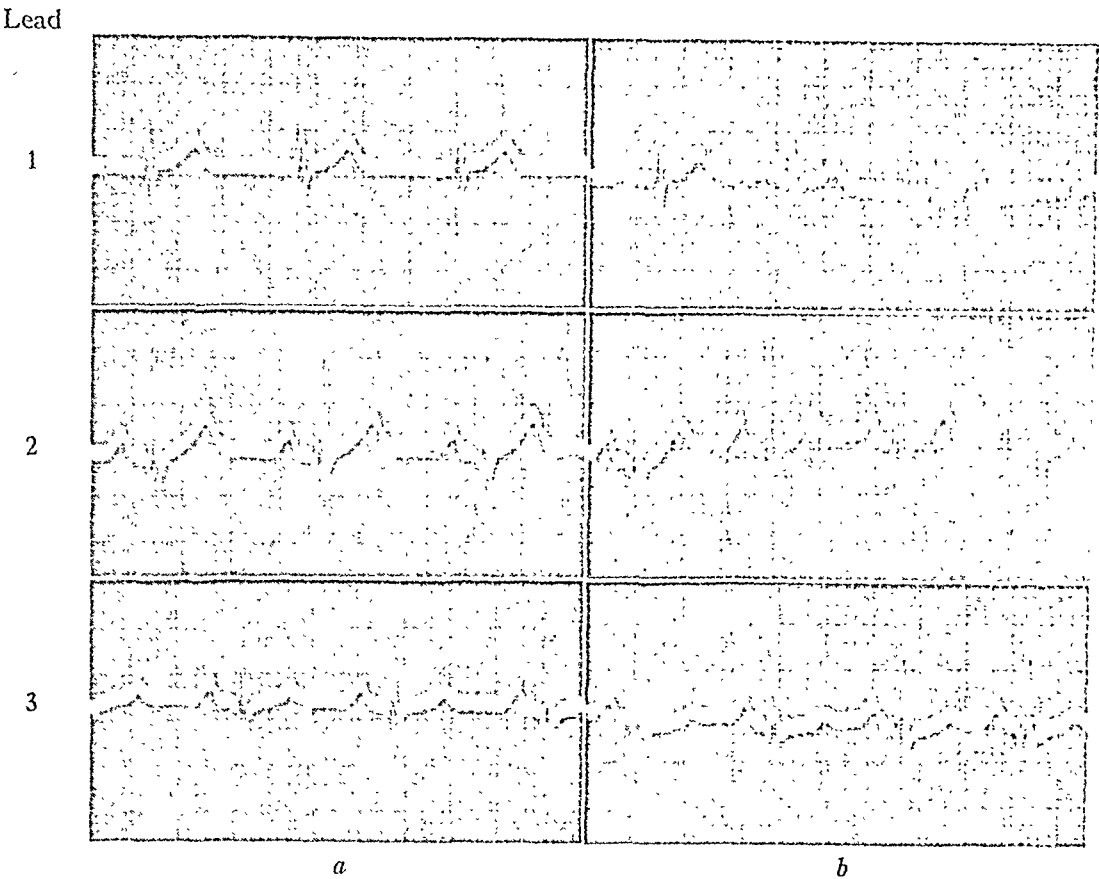


FIG. 1. EFFECT OF EXERCISE
C. H. Column A taken during control period; column B, during exercise, 12 minutes after start. Leads 1, 2, 3.

TABLE 1
Exercise

Subject	J. B. B.		A. S. H.		G. S.		J. R. G.		C. H.	
	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect
Pulse rate	86	140	66	96	66	75	75	100	85	100
Blood pressure	100/70	115/75	132/98	155/90	100/55	125/70	125/90	150/90	95/60	125/60
T-1 (10 ⁻⁴ volt)*	3	2	4	3	3	3	4	3	2.75	2.25
T-2 (10 ⁻⁴ volt)*	2.5	1.75	3.5	2.5	6	5.5	3.5	3	3.5	2.5
T-3 (10 ⁻⁴ volt)*	-0.25	-0.25	-0.1	-0.1	3	2	-0.5	0	1.25	1
QRS axis (degrees by Einthoven triangle)	+38	+38	+45	+40	+75	+80	+42	+85	+78	+70

* This is 10 to the minus fourth power, that is, a tenth of a millivolt.

during exercise. Joffe (9), for example, observed elevation of the T waves in twenty-two normal subjects after exercise. Our findings are in agreement with those of v. Mentzingen (10) who, in studying the electrocardiograms of 451 subjects during exercise, noted lower T waves in 410 of them. Most of his subjects, however, had abnormal hearts. We wish to emphasize that our tracings were taken with the subject actually exercising and that within half a minute after the subject stopped motion, the lowered T waves began to return to (or surpass) their former height.

2. Adrenaline

A. Method. One cc. of a 1:1000 solution of adrenaline hydrochloride was injected subcutaneously. One subject gave a history of marked response to adrenaline, so that 0.5 cc. was given to him. Tracings were taken five, ten, fifteen,

twenty, and thirty minutes after the drug was administered.

B. Results. Adrenaline lowered all the T waves of the three leads in three subjects, while in the other two subjects all the T waves were lowered, except that in one subject T-1 and in the other

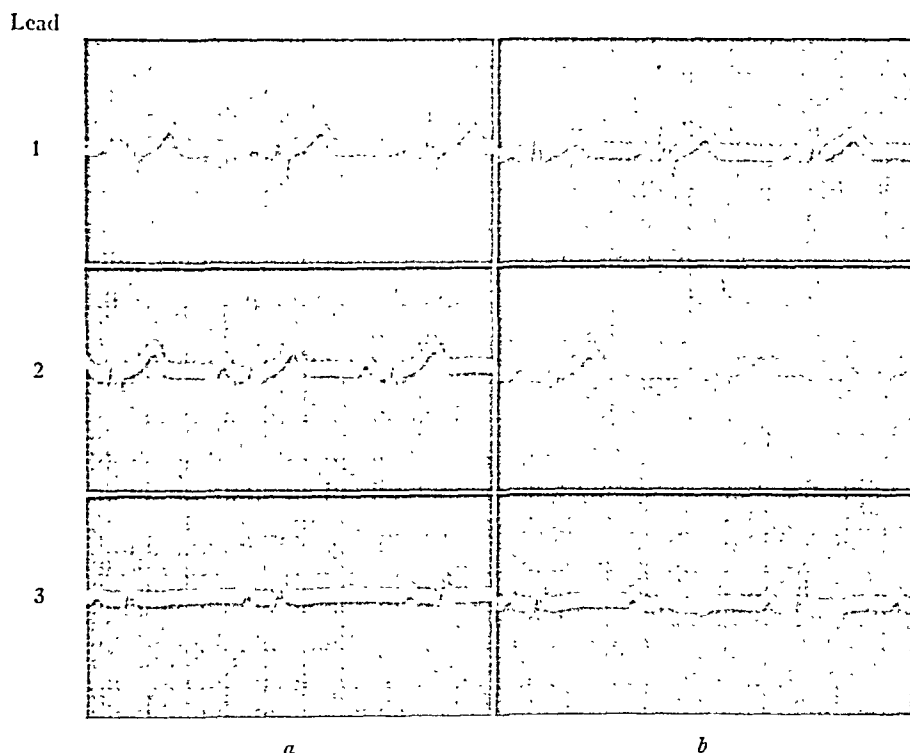


FIG. 2-A. EFFECT OF ADRENALINE

J. R. G. Column A taken during control period; column B, 22 minutes after adrenaline HCl, 1:1000 solution, 1 cc. subcutaneously. Leads 1, 2, 3.

TABLE II

Adrenaline hydrochloride

Subject	J. B. B.		A. S. H.		G. S.		J. R. G.		C. H.	
	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect
Pulse rate	75	92	72	70	60	66	75	86	85	80
Blood pressure	110/70	120/55	130/95	150/80	110/60	145/60	125/80	120/60	95/55	110/50
T-1	2.5	2	2.5	2	2.5	2.5	3	2	2.25	2
T-2	3	2.25	2	1.5	4.75	4	3	2	3.5	2
T-3	-0.5	-0.5	-1	-1.5	2.5	2	0	-1	1	0.5
QRS axis	+38	+42	+50	+50	+68	+68	+70	+70	+75	+68

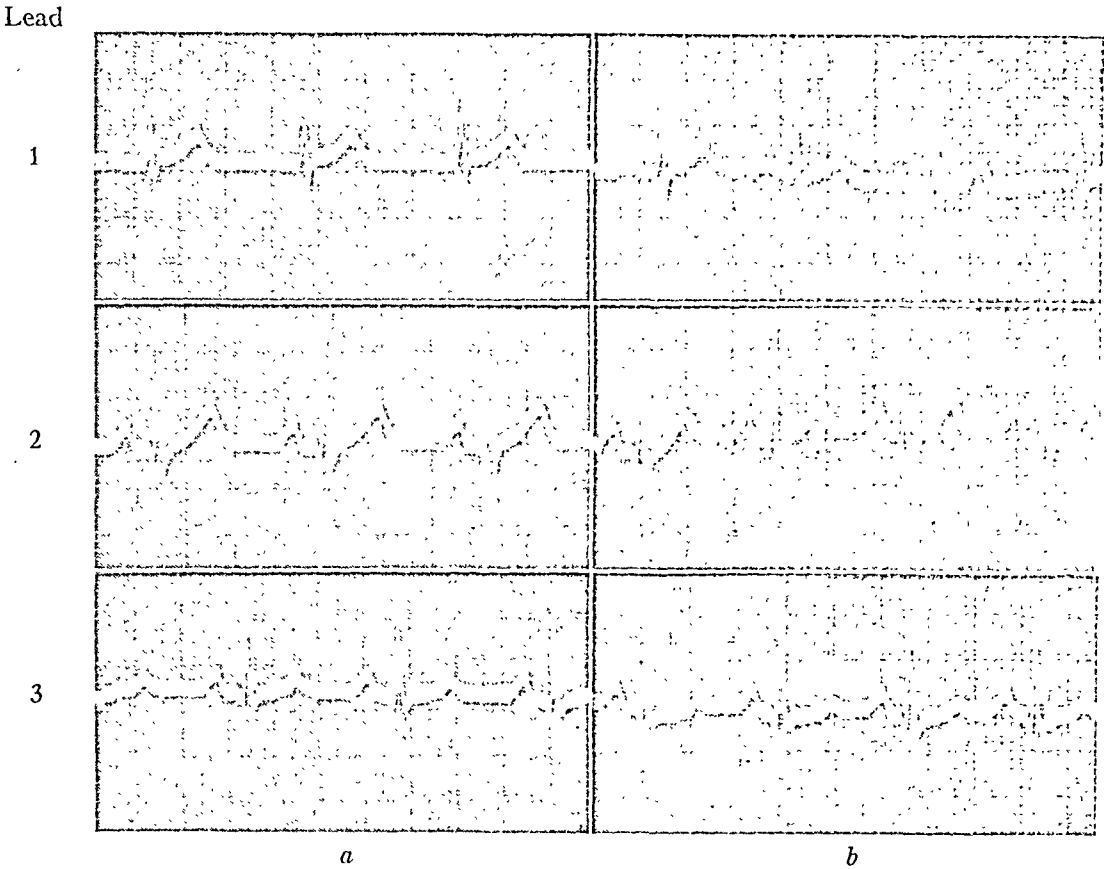


FIG. 1. EFFECT OF EXERCISE

C. H. Column A taken during control period; column B, during exercise, 12 minutes after start. Leads 1, 2, 3.

TABLE 1
Exercise

Subject	J. B. B.		A. S. H.		G. S.		J. R. G.		C. H.	
	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect
Pulse rate	86	140	66	96	66	75	75	100	85	100
Blood pressure	100/70	115/75	132/98	155/90	100/55	125/70	125/90	150/90	95/60	125/60
T-1 (10 ⁻⁴ volt)*	3	2	4	3	3	3	4	3	2.75	2.25
T-2 (10 ⁻⁴ volt)*	2.5	1.75	3.5	2.5	6	5.5	3.5	3	3.5	2.5
T-3 (10 ⁻⁴ volt)*	-0.25	-0.25	-0.1	-0.1	3	2	-0.5	0	1.25	1
QRS axis (degrees by Einthoven triangle)	+38	+38	+45	+40	+75	+80	+42	+85	+78	+70

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B. Results. Adrenaline lowered all the T waves of the three leads in three subjects, while in the other two subjects all the T waves were lowered, except that in one subject T-1 and in the other

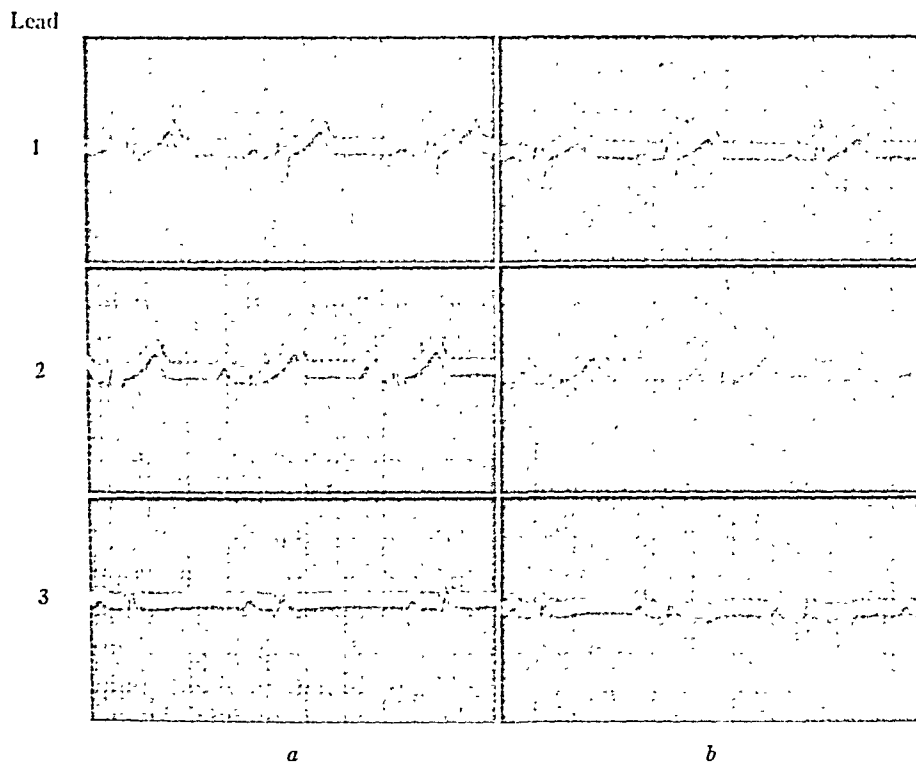


FIG. 2-A. EFFECT OF ADRENALINE

J. R. G. Column A taken during control period; column B, 22 minutes after adrenaline HCl, 1:1000 solution, 1 cc. subcutaneously. Leads 1, 2, 3.

TABLE II

Adrenaline hydrochloride

Subject	J. B. B.		A. S. H.		G. S.		J. R. G.		C. H.	
	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect
Pulse rate	75	92	72	70	60	66	75	86	85	80
Blood pressure	110/70	120/55	130/95	150/80	110/60	145/60	125/80	120/60	95/55	110/50
T-1	2.5	2	2.5	2	2.5	2.5	3	2	2.25	2
T-2	3	2.25	2	1.5	4.75	4	3	2	3.5	2
T-3	-0.5	-0.5	-1	-1.5	2.5	2	0	-1	1	0.5
QRS axis	+38	+42	+50	+50	+68	+68	+70	+70	+75	+68

T-3 showed no change (Figure 2-A and Table II). The effect was maximal in ten to fifteen minutes. One subject was given 1 cc. of a 1:100,000 solution of adrenaline intravenously. This resulted in marked lowering of the T wave, with transient inversion; Lead 2 only was recorded in this experiment (Figure 2-B). One subject showed frequent ventricular premature beats, while another showed a varying P-R interval with inverted P waves for a few minutes after adrenaline. Arrhythmias under adrenaline have been noted by others (11).

C. *Comment.* Our finding of lowered T waves due to adrenaline is in agreement with that of Clough (12), who studied the effect of 7.5 minims of 1:1000 adrenaline intramuscularly in normal males and in subjects with irritable hearts. He found a decrease of 1 to 2 mm. in the T waves. Levine et al. (13) gave the same dose as we to ten normals and stated that "in young adults there was an average fall in the T wave amplitude in Lead 2 of 0.8 mm., five cases showing an in-

crease from 0.4 to 1.5 mm. and five a decrease of from 1.5 to 3.3 mm."

3. *Ergotamine tartrate*

A. *Method.* One cc. of ergotamine tartrate (1 mgm.) was given subcutaneously to two subjects, but, due to marked nausea in those cases, only 0.5 cc. (0.5 mgm.) was given to the others. Tracings were taken five, fifteen, thirty, and, in three subjects, sixty minutes after the drug was given.

B. *Results.* Ergotamine raised all the T waves of the three leads of five subjects, except that T-2 in one subject showed no change¹ (Figure 3 and Table III). The effect was maximal in thirty to sixty minutes.

C. *Comment.* Almost identical results have been obtained by Nordenfelt (14) with twenty normals, using 1 cc. (1 mgm.). There is considerable evidence that ergotamine opposes the action of adrenaline (8, 15, 16, 17). We have observed that exercise, which stimulates the sympathetic nervous system, and adrenaline, lower the T waves, and therefore the opposite effect of raising the T waves was of considerable interest. The elevation of the T waves was obtained without marked slowing of the pulse.

4. *Atropine sulfate*

A. *Method.* One-fiftieth of a grain of atropine sulfate was given subcutaneously. Marked dryness of the mouth was noted in each subject after about forty minutes. Tracings were taken twenty, forty, sixty and ninety minutes after the drug was given.

B. *Results.* Atropine lowered all the T waves of the three leads in three subjects. It lowered all the T waves except T-1 in one subject and all but T-3 in another subject (Figure 4 and Table IV). One subject developed A-V nodal rhythm twenty minutes after the atropine injection. By forty minutes this had changed to sinus tachycardia. Wilson (18) and Lewis (19) have noted this as an early action of atropine in normals.

C. *Comment.* Atropine significantly lowers the T waves, as shown by two of us already in a previous paper (1). Its action is to inhibit the parasympathetic nervous system, particularly the vagus.

¹ In this case T-1 and T-3 showed only minute changes.

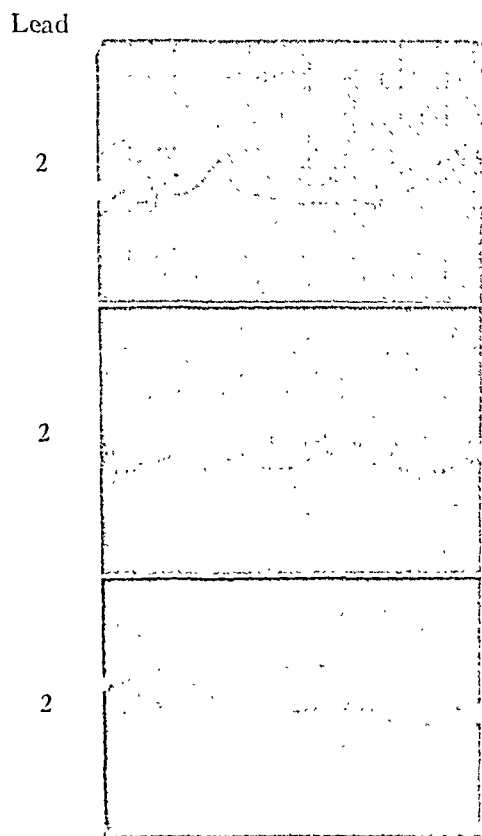


FIG. 2-B. EFFECT OF ADRENALINE

A. S. H. During administration of adrenaline HCl, 0.01 mgm. (1 cc. of 1:100,000 solution) intravenously; control, beginning effect, maximal effect. Leads 2, 2, 2.

TABLE III
Ergotamine tartrate

Subject	J. B. B.		A. S. H.		G. S.		J. R. G.		C. H.	
	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect
Pulse rate	75	66	66	66	66	50	85	66	75	66
Blood pressure	115/70	105/80	135/85	148/108	100/60	100/60	120/70	110/80	95/60	90/60
T-1	2.5	3	2.5	4	2	2.5	3.25	4.5	2	3.25
T-2	3.5	3.5	1.5	2.5	3.5	6.5	3.25	4.5	2.5	4.5
T-3	0.5	1	-1	0.5	2	3.75	-1.5	-0.75	0.5	2
QRS axis	+44	+48	+42	+42	+72	+78	+64	+60	+60	+72

Lead

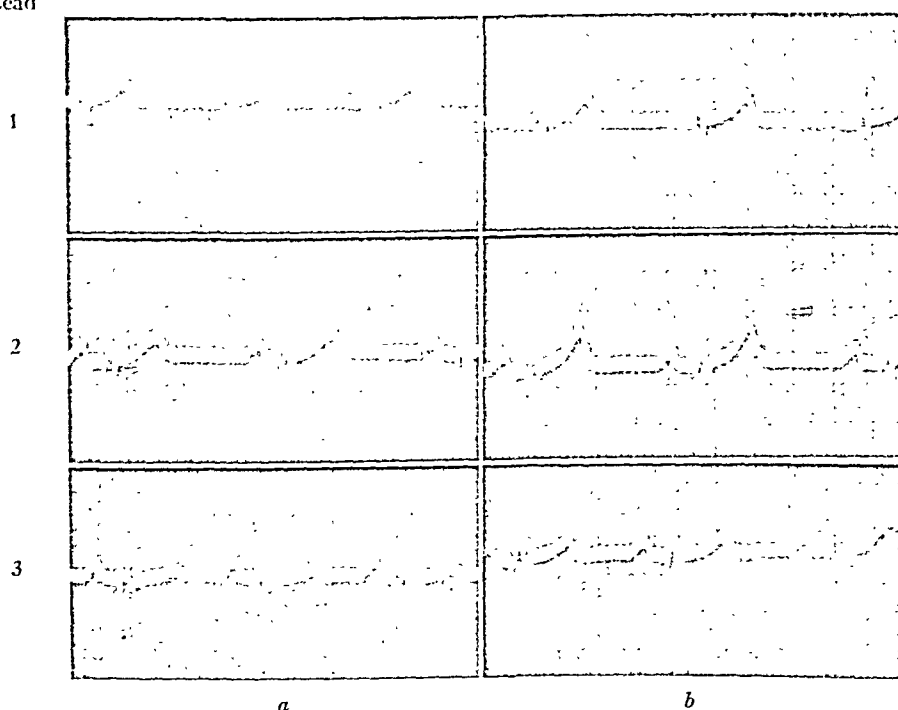


FIG. 3. EFFECT OF ERGOTAMINE

C. H. Column A taken during control period; column B, 45 minutes after ergotamine tartrate, 0.5 mgm. subcutaneously. Leads 1, 2, 3.

Thus drugs which increase sympathetic tone or inhibit parasympathetic activity lower the T waves, while those which lower sympathetic tone, such as ergotamine, raise the T waves.

5. Mecholyl

A. Method. Two subjects received 25 mgm. of mecholyl subcutaneously, but the general reaction

was so marked that 15 mgm. was given to the others. The latter group obtained a satisfactory response with sweating, salivation, diffuse blushing, and, as has been noted by many others, tachycardia.

B. Results. Mecholyl lowered all the T waves in the three leads of all five subjects. All subjects developed tachycardia, beginning one to one and

Lead

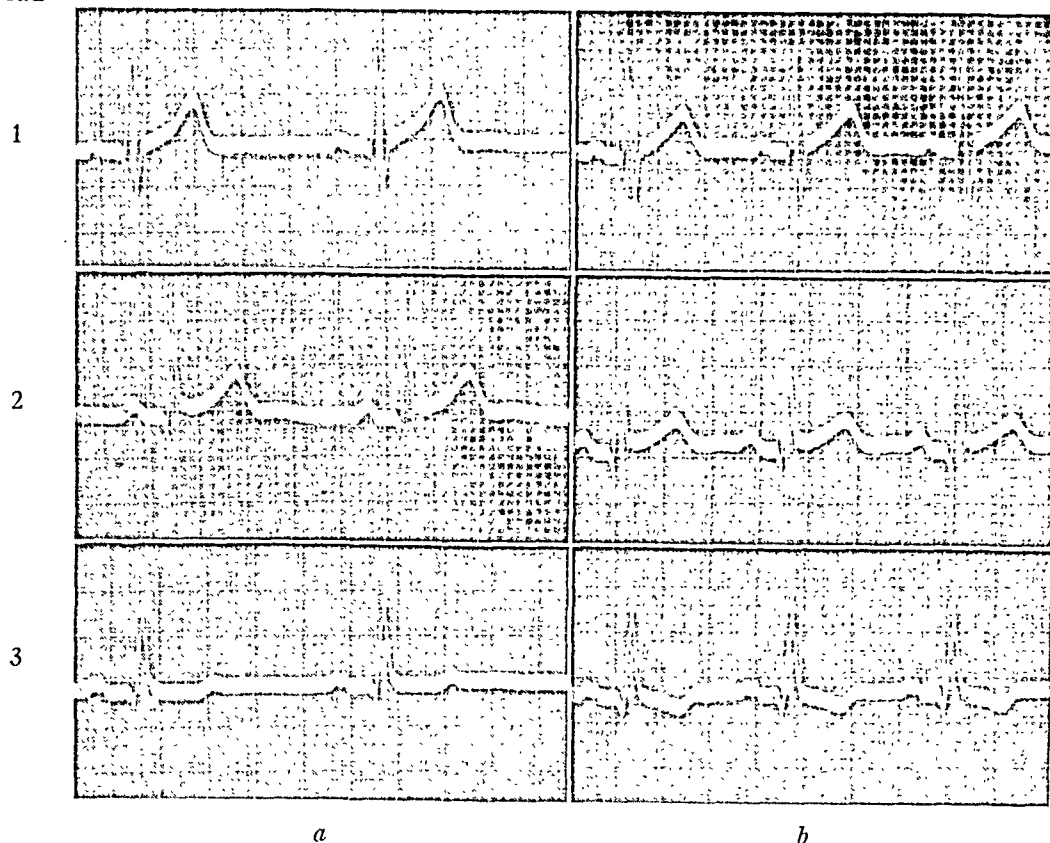


FIG. 4. EFFECT OF ATROPINE

A. S. H. Column A taken during control period; column B, 60 minutes after atropine sulphate, 1.3 mgm. (1/50 gr.) subcutaneously. Leads 1, 2, 3.

TABLE IV
Atropine sulfate

Subject	J. B. B.		A. S. H.		G. S.		J. R. G.		C. H.	
	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect
Pulse rate	86	100	60	86	66	86	70	86	85	100
Blood pressure	115/70	100/60	120/88	140/95	100/60	100/60	120/75	110/70	100/60	90/55
T-1	2.5	1.5	6	4	2.5	2	3.5	3	1.75	2
T-2	3	2	5	2.5	4.5	3	4.5	4	3	2.75
T-3	0.25	0	1	-1.5	2.25	1	0.25	0.75	1.5	1
QRS axis	+50	+58	+58	+58	+74	+74	+58	+70	+72	+84

a half minutes after administration. Four subjects showed a fall in blood pressure (Figure 5 and Table V).

C. Comment. Mecholyl, supposedly a parasympathetic stimulant par excellence, has been found to cause tachycardia by all its users (20 to 24). This seemingly paradoxical effect on the heart

has been discussed ably by others (23). Rothberger (25), giving acetyl choline intravenously to cats, noted an initial transient slowing followed by tachycardia. Accordingly, we took continuous tracings of Lead 2, starting before injection of mecholyl and continuing until the tachycardia was well started, but observed no preliminary slowing

Lead

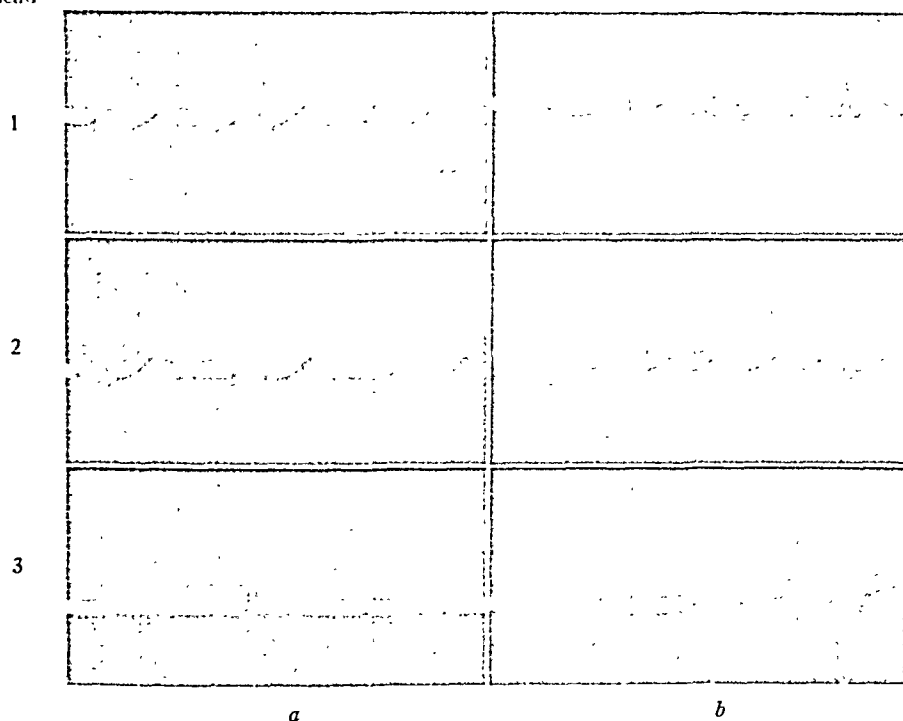


FIG. 5. EFFECT OF MECHOLYL

J. R. G. Column A taken during control period; column B, 2 minutes after mecholyl, 15 mgm. subcutaneously. Leads 1, 2, 3.

TABLE V
Mecholyl

Subject	J. B. B.		A. S. H.		G. S.		J. R. G.		C. H.	
	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect	Control	Maximum effect
Pulse rate	86	120	60	140	66	90	75	110	85	100
Blood pressure	115/70	120/70	135/90	150/95	100/60	100/60	120/70	110/60	100/60	80/50
T-1	2.5	2	4	1.5	2	1.5	2.5	1.5	2.5	1.75
T-2	2	1	3	0	4.5	3	2.5	1.25	2.75	2.5
T-3	0	-0.25	-1.5	-3	2.5	1.75	0.25	0	1	0.75
QRS axis	+50	+50	+60	+90	+75	+70	+70	+75	+72	+85

of the heart rate. We are unable to explain the paradoxical tachycardia and lowering of the T waves.

6. Right carotid sinus pressure

A. Method. This was tried on four subjects. No observations were made on the blood pressure in these experiments.

B. Results. All subjects showed slowing of the pulse. There was an increase of the amplitude of all the T waves in Lead 3 of the five subjects. The T waves in Lead 1 and Lead 2 were elevated in two of the subjects. One Lead 1 showed a decrease in the T wave (Table VI).

C. Comment. The predominant action of carotid sinus pressure on the electrocardiogram, aside

TABLE VI
Right carotid sinus pressure

Subject	J. B. B.		A. S. H.		J. R. G.		C. H.	
	Con- trol	Maxi- mum effect	Con- trol	Maxi- mum effect	Con- trol	Maxi- mum effect	Con- trol	Maxi- mum effect
Pulse rate	86	77	70	55	75	66	85	66
T-1	3	3	3.5	4	4	3.5	2.75	3
T-2	2.5	3	3	4.5	3.5	3.5	3	3
T-3	-0.5	0.25	-1	0	-0.5	0	1	1.25
QRS axis	+38	+40	+45	+45	+42	+50	+78	+75

from slowing of the rate, is to raise the T waves. These results may be considered as agreeing with the concept that factors which inhibit sympathetic tone or increase the vagal tone raise the T waves.

DISCUSSION

The vague conceptions in the minds of most physicians, both in general and in particular, about the effects of these various procedures and of autonomic nervous influences on the electrocardiogram, will, we hope, be clarified by this report. The importance of our findings is also of a practical nature. For instance, if a "repeat" electrocardiogram is taken on a patient who receives atropine, or adrenaline, or mecholyl, shortly before the second tracing, T wave changes would be noted and might be interpreted as a change in the basic cardiac status, when in reality they are due to the effect of the drug. These drugs are commonly used in hospital practice and it was in part for this reason that the present study was made.

Interesting speculations have been mentioned regarding the sympathetic and parasympathetic nervous systems and the part they may play in affecting the electrocardiogram. Of the exact nature of the part they play in these results, we are not sure. Indeed, in the intact normal subject, compensatory mechanisms probably play important roles in determining the final result. Our findings suggest, however, that adrenergic factors lower the T waves and cholinergic factors raise them. The anomalous action of mecholyl has been discussed. It may also be postulated from our results that procedures which elevate the pulse rate, lower the T waves, and *vice versa*. That this is true, there is no doubt. However, in some of the subjects with no change in pulse rate, the

effect on the electrocardiogram was no less marked. This was particularly true in the case of adrenaline and ergotamine.

SUMMARY

1. Exercise lowers the T waves of the normal human electrocardiogram, with return toward normal in less than a minute. During recovery the amplitude of T may be greater than normal.
2. Adrenaline lowers the T waves. The effect lasts from fifteen to thirty minutes.
3. Ergotamine tartrate raises the T waves. This effect lasts as long as an hour.
4. Atropine lowers the T waves. The effect is maximal in one hour but may last ninety minutes.
5. Mecholyl lowers the T waves and causes tachycardia without preliminary bradycardia.
6. Right carotid sinus pressure causes an elevation of the T waves.
7. The importance of taking these changes into account when interpreting electrocardiograms is stressed.

BIBLIOGRAPHY

1. Graybiel, A., Starr, R. S., and White, P. D., Electrocardiographic changes following the inhalation of tobacco smoke. *Am. Heart J.*, 1938, 15, 89.
2. Barker, P. S., Shrader, E. L., and Ronzoni, E., The effects of alkalosis and of acidosis upon the human electrocardiogram. *Am. Heart J.*, 1939, 17, 169.
- 3a. Scherf, D., and Weissberg, J., The alterations of the T-waves caused by a change of posture. *Am. J. Med. Sc.*, 1941, 201, 693.
- b. White, P. D., Chamberlain, F. L., and Graybiel, A., Inversion of the T waves in Lead II caused by a variation in position of the heart. *Brit. Heart J.*, 1941, 3, 233.
4. Douglas, A. H., Gelfand, B., and Shookhoff, C., Production by epinephrine of S-T changes in the electrocardiogram of the cat, similar to those of coronary occlusion. *Am. Heart J.*, 1937, 14, 211.
5. Milles, G., and Smith, P. W., Effects of epinephrine on the heart. *Am. Heart J.*, 1937, 14, 198.
6. Bartos, E., and Burstein, J., Can variations in ventricular systole be determined from electrocardiogram deflections? *J. Lab. and Clin. Med.*, 1924, 9, 217.
7. Hoff, H. E., and Nahum, L. H., The role of adrenaline in the production of ventricular rhythms and their suppression by acetyl- β -methycholine chloride. *J. Pharmacol. and Exper. Therap.*, 1934, 52, 235.
8. Youmans, J. B., and Trimble, W. H., Experimental and clinical studies of ergotamine; the effect of ergotamine on the heart rate of trained unanest-

- thetized dogs. *J. Pharmacol. and Exper. Therap.*, 1930, 38, 133.
9. Joffe, E., La modification de l'électrocardiogramme humain par l'effort. *Compt. rend. Soc. de biol.*, 1938, 128, 809.
10. v. Mentzingen, A., Die Bedeutung der Veränderung der Nachschwankung des Elektrokardiogramms nach Belastung für die Beurteilung der Funktion des Herzens. *Klin. Wchnschr.*, 1934, 13, 88.
11. Hume, W. E., The action of adrenalin chloride on the human heart. *Quart. J. Med.*, 1928, 21, 459.
12. Clough, H. D., Studies on Epinephrin III; Effect of epinephrin on the electrocardiograms of patients with "irritable heart." *Arch. Int. Med.*, 1919, 24, 284.
13. Levine, S. A., Ernstenc, A. C., and Jacobson, B. M., The use of epinephrine as a diagnostic test for angina pectoris. *Arch. Int. Med.*, 1930, 45, 191.
14. Nordenfelt, O., Die Ekg-Veränderungen bei orthostatischen Kreislaufstörungen und Ergotamintartrat. *Ztschr. f. Kreislauforsch.*, 1939, 31, 761.
15. Kolm, R., and Pick, E. P., Über die Bedeutung des Calciums für die Erregbarkeit der sympathischen Herznervenendigungen. *Arch. f. d. ges. Physiol.*, 1921, 189, 137.
16. Jang, C-S., The potentiation and paralysis of adrenergic effects by ergotoxine and other substances. *J. Pharmacol. and Exper. Therap.*, 1941, 71, 87.
17. Goodman, L., and Gilman, A., *The Pharmacological Basis of Therapeutics*. The Macmillan Co., New York, 1941, p. 340.
18. Wilson, F. N., The production of atrioventricular rhythm in man after the administration of atropin. *Arch. Int. Med.*, 1915, 16, 989.
19. Lewis, Sir Thomas, *The Mechanism and Graphic Registration of the Heart Beat*. Shaw and Sons, Ltd., London, 1925, p. 196, 3rd edition.
20. Starr, I., Elsom, K. A., Reisinger, J. A., and Richards, A. N., Acetyl- β -methylcholin; the action on normal persons with note on the action of ethyl ether of β -methylcholin. *Am. J. M. Sc.*, 1933, 186, 313.
21. Page, I. H., Acetyl- β -methylcholine (mecholin). Observations concerning its action on blood pressure, skin temperature, and heart, as exhibited by the electrocardiogram of hypertensive patients. *Am. J. M. Sc.*, 1935, 189, 55.
22. Kovacs, J., Saylor, L. L., and Wright, I. S., The pharmacological and therapeutic effects of certain choline compounds. *Am. Heart J.*, 1936, 11, 53.
23. Dameshek, W., Loman, J., and Myerson, A., Human autonomic pharmacology; the effect on the normal cardiovascular system of acetyl- β -methylcholine chloride, atropine, prostigmin and benzedrine—with especial reference to the electrocardiogram. *Am. J. M. Sc.*, 1938, 195, 88.
24. Weiss, S., and Ellis, L. B., The comparative effects of the intravenous administration to man of acetylcholine and acetyl- β -methylcholine. *J. Pharmacol. and Exper. Therap.*, 1934, 52, 113.
25. Goldenberg, M., and Rothberger, C. J., Über die Wirkung von Acetylcholin auf das Warmblüterherz. *Ztschr. f. d. ges. exper. Med.*, 1934, 94, 151.

ABSENCE OF BENEFICIAL EFFECTS FROM INJECTIONS OF DESOXYCORTICOSTERONE ACETATE AND OF CORTICAL ADRENAL EXTRACT IN EXPERIMENTAL ANURIA¹

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Hoff, Smith and Winkler (1) found that dogs with anuria secondary to nephrectomy or to ureteral ligation die from cardiac arrest due to auto-intoxication with potassium. Potassium, derived from the breakdown of the animal's own tissues, can apparently neither be excreted nor stored, and gradually accumulates until a concentration sufficient to cause cardiac arrest is attained. A clinical state somewhat analogous to these forms of experimental anuria has recently been described by Beall and others (2, 3, 4, 5) in patients with anuria of several days duration following crushing injuries. In some patients the potassium of serum rose considerably, and it seems possible that in some instances death may have been due to potassium poisoning. Since desoxycorticosterone acetate (hereafter called "DOCA") may, under certain circumstances, lower the serum potassium, the possibility that this substance might prove of therapeutic value naturally presented itself. Selye (6, 7) and Dosne (8) have in fact reported considerable prolongation of life in anuric rats previously treated with DOCA, and have termed this effect an "antiuremic action." The present study deals mainly with the results of DOCA injections in anuric dogs. Several supplementary experiments with whole cortical extract were carried out.

MATERIALS AND METHODS

The general procedure and the specific techniques employed have been described in detail previously (1). No water or food was given during the course of the experiments, so that vomiting with secondary chloride depletion was virtually absent. All injections of DOCA or of cortical extract were made *after* the ureters were ligated.

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Fourteen dogs received DOCA intramuscularly or subcutaneously in varying amounts, while four others received cortical extract. Anuria was produced in all instances by ureteral ligation. The course of these injected dogs is compared with that of fifteen dogs in a control series; the protocols of twelve of these have already been published (1). The DOCA was usually dissolved in propylene glycol with gentle warming, injections then being made every twelve to twenty-four hours, in order that some active substance might continually be present. In two experiments the regular commercial preparation of DOCA dissolved in sesame oil was employed.²

RESULTS

The protocols are summarized in Table I. The times of survival after ligation and the concentrations of potassium in serum at death fall within the same ranges in the injected animals and in those of the control series. The several groups are therefore essentially the same in each of these two respects. Also the increase in serum potassium, relative to the increase in the blood non-protein nitrogen, is about the same in the control animals and in the injected animals.

Clinically the behavior of the treated animals was indistinguishable from that of the untreated ones. The electrocardiographic changes prior to death in the injected animals were of exactly the same character, typical of progressive potassium poisoning, as those of the control group. The usual relationship of serum potassium to electrocardiographic changes (1, 9) was unaltered.

DISCUSSION

Obviously DOCA had no beneficial effect whatsoever in these animals. Yet DOCA, adminis-

² Dr. E. Schwenk, of the Research Division of Schering Corporation, generously supplied us with all the desoxycorticosterone acetate used in these studies. Commercial whole adrenal cortical extract (Wilson) was used in three experiments. In a fourth a special adrenal cortical extract prepared by Dr. E. C. Kendall, and kindly furnished to us by him, was employed.

TABLE I

Summary of results

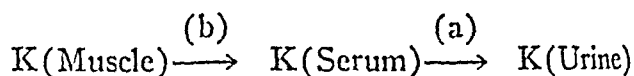
All injections given *after* ligation of ureters

Number	Dose	Blood NPN		Potassium of serum		Survival time
		Initial	Death	Initial	Death	
	mgm. ¹ or cc. ²	mgm. per cent		mM. per liter		hours
(A) EXPERIMENTS WITH DESOXYCORTICOSTERONE ACETATE						
1	6+10+10	38	286	6.3	13.2	90
2	10+10+10	29	247	6.6	10.1	108
3	5+5+5+5	36	337	5.2	14.4	102
4	25+15	29	282	4.3	12.0	66
5	50+25	33	222	4.3	10.2	47
6	50+25	29	291	3.7	10.8	72
7	25+25+10+10	41	277	7.4 ³	10.6	77
8	25+25+10+10	28	242	5.4 ³	17.7	76
9 ⁴	25+25+10+10	106	325	6.4 ³	11.8	78
10	100+33+35	28	335	3.2	10.7	79
11	100+33+35	25	288	3.5	11.1	90
12	100+33+35	29	274	3.8	12.2	84
13 ⁵	50+(15×6)	32	344	4.9	11.5	86
14 ⁵	50+(15×5)	29	250	6.2	12.5	82
(B) EXPERIMENTS WITH CORTICAL EXTRACT (WILSON)						
1	90		370	5.0	10.8	114
2	90	36	351	4.1	16.5	96
3	70	46	298	4.7	13.1	72
4	100 ⁶	24	265	4.4	12.0	77
(C) CONTROL EXPERIMENTS: AVERAGE VALUES FROM FIFTEEN EXPERIMENTS (1)						
	none	30±4	290±65	5.4±0.8	13.8±2.9	102±24

¹ Individual figures refer to doses every 12 to 24 hours.² 10 cc. every 12 hours.³ Just after operation.⁴ Chronic nephritis at autopsy.⁵ DOCA in sesame oil.⁶ Dr. Kendall's extract used.

tered parenterally to the intact animal in doses comparable to those given here, is known to cause an increased renal excretion of potassium (10). In fact, if potassium intake be restricted, DOCA injection in the normal animal will in time deplete the potassium of serum and of muscle as well (11). Since this increased urinary excretion is associated with a diminished concentration of serum potassium, it is not simply a reflection of an extrarenal disturbance of potassium metabolism. A specific effect of DOCA upon the normal kidney therefore seems well established. Our experiments indicate that this renal action is responsible for the main disturbing effect of DOCA upon potassium metabolism, since potassium distribution is not demonstrably affected by it in the anuric animal. Expressed graphically, DOCA injection in the intact animal accelerates the fol-

lowing sequence:



DOCA certainly does act at "(a)." It has, however, been suggested that DOCA and cortical extract may act at "(b)" as well (12). Only some such hypothesis would justify the conclusion that DOCA had a beneficial action in the treatment of anuria. Our experiments do not support the validity of this second supposition.

It is not proper, on the basis of these experiments, to deny extrarenal action of DOCA or of cortical extract upon potassium metabolism. On the contrary, there is good evidence that cortical extracts rich in "compound E" (11 dehydro-17 hydroxy corticosterone) accelerate protein breakdown (13), which in turn might accelerate the release of cellular potassium. Such extracts may also increase glycogen deposition in the liver (14), a process which is thought to be associated with the withdrawal of some potassium from the extracellular fluid (15). All that can be said is that such effects are quantitatively too small to be detected under the conditions of our experiments.

It has been mentioned before that Selye and Dosne (6, 7, 8) have found that rats previously treated with DOCA survive ureteral ligation for a longer time than do those not so treated. Actually, there is no experimental conflict between their observations and ours. They gave the DOCA injections for a period *before* the production of the anuria, whereas our injections were always given *afterwards*. By giving DOCA while the kidneys were still functionally intact, they encouraged an excessive potassium excretion, which might in turn be expected to produce some measure of depletion of tissue potassium (11). Durlacher and Darrow (16) have found that rats depleted of potassium, whether by a low potassium diet or by DOCA administration, will survive longer than control animals, following ureteral ligation. This is presumably due to the presence of an abnormally large potential reservoir in which the potassium, produced by the breakdown of their own tissues after the establishment of anuria, may be stored. A longer time would, therefore, be required for such animals to build up a concentration of potassium in the serum sufficient to bring about cardiac arrest. The prac-

tical therapeutic possibilities inherent in such use of DOCA in "uremia" are obviously very restricted.

SUMMARY AND CONCLUSIONS

1. In dogs with anuria following ureteral ligation, the subcutaneous or intramuscular injection of desoxycorticosterone acetate and of cortical adrenal extract were completely without beneficial effect.

2. No effect on potassium distribution within the body was detected.

3. Failure to demonstrate such an action of desoxycorticosterone acetate upon potassium distribution in the anuric animal suggests that its effects upon potassium metabolism in the intact animal are secondary to its renal action.

4. On the basis of present knowledge of its pharmacological action, desoxycorticosterone acetate could only be expected to influence the course of an anuric subject favorably if it were given prior to the establishment of anuria.

BIBLIOGRAPHY

1. Hoff, H. E., Smith, P. K., and Winkler, A. W., The cause of death in experimental anuria. *J. Clin. Invest.*, 1941, 20, 607.
2. Bywaters, E. G. L., and Beall, D., Crush injuries with impairment of renal function. *Brit. M. J.*, 1941, 1, 427.
3. Beall, D., Bywaters, E. G. L., Belsey, R. H. R., and Miles, J. A. R., Crush injury with renal failure. *Brit. M. J.*, 1941, 1, 432.
4. Mayon-White, R., and Solandt, O. M., A case of limb compression ending fatally in uraemia. *Brit. M. J.*, 1941, 1, 434.

5. Editorial: Crush injury and renal function. *Brit. M. J.*, 1941, 1, 445.
6. Selye, H., The beneficial action of desoxycorticosterone acetate in uraemia. *Canad. Med. Ass. J.*, 1940, 43, 333.
7. Selye, H., and Nielsen, K., Action of desoxycorticosterone on non-protein nitrogen content of blood during experimental uremia. *Proc. Soc. Exper. Biol. and Med.*, 1941, 46, 541.
8. Dosne, C., The effect of dosage and duration of administration on the anti-uremia effect of desoxycorticosterone. *Am. J. Physiol.*, 1941, 134, 71.
9. Winkler, A. W., Hoff, H. E., and Smith, P. K., Electrocardiographic changes and concentration of potassium in serum following injection of potassium chloride. *Am. J. Physiol.*, 1938, 124, 478.
10. Kuhlmann, D., Ragan, C., Ferree, J. W., Atchley, D. W., and Loeb, R. F., Toxic effects of desoxycorticosterone esters in dogs. *Science*, 1939, 90, 496.
11. Miller, H. C., and Darrow, D. C., Relation of serum and muscle electrolyte, particularly potassium, to voluntary exercise. *Am. J. Physiol.*, 1941, 132, 801.
12. Ingle, D. J., Nilson, H. W., and Kendall, E. C., The effect of cortin on the concentrations of some constituents of the blood of adrenalectomized rats. *Am. J. Physiol.*, 1937, 118, 302.
13. Wells, B. B., and Kendall, E. C., A qualitative difference in the effects of compounds separated from the adrenal cortex on distribution of electrolytes and on atrophy of the adrenal and thymus glands of rats. *Proc. Staff Meet., Mayo Clin.*, 1940, 15, 133.
14. Long, C. N. H., Katzin, B., and Fry, E. G., The adrenal cortex and carbohydrate metabolism. *Endocrinology*, 1940, 26, 309.
15. Fenn, W. O., The deposition of potassium and phosphate with glycogen in rat livers. *J. Biol. Chem.*, 1939, 128, 297.
16. Durlacher, S., and Darrow, D. C. (To be published.)

PROTECTIVE ACTIVITY OF NORMAL HUMAN AND ANIMAL SERA FOR SULFAPYRIDINE-TREATED MICE INFECTED WITH PNEUMOCOCCI

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With the belief that the therapeutic activity of sulfonamide drugs in pneumococcal infections depends in part on the presence of protective mechanisms other than the bacteriostasis produced by the drugs, a knowledge of the enhancement offered by normal serum is needed to clarify the mechanisms of this therapeutic action. The purpose of the present communication is to present experiments which deal with the activity of normal animal and human sera in enhancing the anti-pneumococcal action of sulfapyridine. Normal mouse, rabbit, guinea pig, rat, dog, infant human, and adult human sera were examined, with white mice used as test animals.

MATERIAL AND METHODS

The test to determine the enhancement of mouse protection produced by each serum consisted of the inoculation of graded doses of pneumococci to three series of mice, one series receiving serum alone, one series receiving sulfapyridine, and one series receiving both serum and sulfapyridine.

The sulfapyridine was administered orally, mixed with the food. The food used was ordinary mouse biscuits, ground in a meat grinder and sifted through a fine sieve. The resulting powder was thoroughly mixed with sulfapyridine in the proportion of one part of the drug to ninety-nine parts of the powdered food, giving a one per cent sulfapyridine content. The mice were housed 6 in a cage, and the powdered food, with the sulfapyridine, given in open boxes, so that they had free access to it.

The maximum protection with sulfapyridine alone resulted from its administration for from 3 to 6 days. There was approximately 33 per cent survival for Type I and survival varied from 20 per cent through 80 per cent with strains of Type III pneumococci. However, only 2-day sulfapyridine treatment was used in enhancement tests because comparisons were most clear cut with sulfapyridine dosage of this duration.

The virulence of the cultures was such that 10^{-4} ml. of a freshly passed, 18-hour blood broth culture killed a mouse weighing 18 to 20 grams within 24 to 48 hours. Varying amounts of culture, 10^{-4} , 10^{-5} , 10^{-6} (1, 10 and 1,000 M.L.D.), were employed in the tests with Type I, and 10^{-4} to 10^{-7} ml. (1, 10, 100, 1,000, 10,000 and 100,000 M.L.D.), with Type III pneumococci. Dilutions were so

adjusted that 0.5 ml. of each contained the desired amount of culture. The culture and serum were injected intraperitoneally, the serum immediately before the culture (within an interval of a few seconds).

The dose of serum for each mouse was 0.5 ml., injected intraperitoneally.

Surviving mice were discharged at the end of 7 days.

Absorption tests were conducted in the following manner: Freshly passed cultures were grown for 4 hours at 37° C. The cultures were killed by heating at 55° C. for 30 minutes, centrifuged at high speed for one hour, and the "supernatant" discarded. The serum was added to the cells in the proportion of 1 ml. of serum to the sediment of 2 ml. of culture. The serum and cells were well mixed and placed in a water bath at 45° C. for one hour. Serum without cells was similarly kept in a water bath at 45° C. for one hour and used as controls. Then 0.1 per cent formalin was added to the mixtures and the tubes placed in an icebox overnight. The serum and cell suspension was then centrifuged and the clear serum withdrawn. Absorbed sera were not used in the protection tests before sterility tests showed complete absence of live organisms. Absorbed sera, without culture, were also injected into mice as additional controls.

RESULTS OF THE MOUSE PROTECTION TESTS

Protection against pneumococcus Type I

Of the 12 normal human adult sera tested against pneumococcus Type I only 2 gave protection when used without sulfapyridine. These sera saved 11 of 12 mice infected with Type I organisms. None of the remaining 10 sera protected the animals to any appreciable extent when administered alone. However, all sera protected the animals to a high degree (from 66 to 100 per cent of the animals tested) when given with sulfapyridine, while the mice receiving sulfapyridine alone either all succumbed to the infection or only one out of 6 mice recovered.

None of the normal infants' sera employed in the tests gave any appreciable protection, either alone or with sulfapyridine, against Type I pneumococcus.

Normal mouse, guinea pig, rabbit, and rat sera did not protect the mice against Type I pneumo-

TABLE I

Protective action of normal human and animal sera on white mice infected with Type I pneumococcus when treated with sulfapyridine

Source of serum	Serum 0.5 ml. alone				Sulfapyridine alone 1 per cent in food for 2 days				Serum and sulfapyridine			
	Number mice tested	Number mice recovered	Per cent of mice recovered	Average days survived	Number mice tested	Number mice recovered	Per cent of mice recovered	Average days survived	Number mice tested	Number mice recovered	Per cent of mice recovered	Average days survived
Adult human 10 samples (None protective when used alone)	60	4	6.6	2.7	60	3	5	3.1	60	46	77	6
Adult human 2 samples (Protective when used alone)	12	11	91.6	5.9	12	1	8.3	3.1	12	12	100	7
Infant human 10 samples 4 individual 6 pooled	30	1	3.3	1.9	36	1	2.7	2.5	31	4	12.9	3.3
Mice 66 pooled	6	0	0	2	6	0	0	2.3	6	1	16.6	4.1
Guinea pigs 6 pooled	6	0	0	1.6	6	0	0	3.3	6	1	16.6	3.3
Rabbit 3 pooled	6	0	0	1.5	6	0	0	2.5	6	0	0	2.8
Dog one	6	4	66.6	5.5	6	1	16.6	3.6	6	6	100	7
Rats 15 pooled	6	0	0	2	6	0	0	2	6	0	0	2.3

coccus when used alone or with sulfapyridine. Normal dog serum, however, gave about 66 per cent protection to the mice when used alone, and 100 per cent protection when used with sulfapyridine.

Table I is a summation of the mouse protection tests, with the various normal sera, against Type I pneumococcus.

As seen from Table II, fresh, unheated human

TABLE II

Mouse protection test with normal human adult serum (757A) used fresh and heated with and without sulfapyridine against Type I pneumococcus

Treatment	Number of M.L.D. of culture	Number of mice infected	Number of mice recovered	Per cent recovered	Average days survived
Fresh serum alone	1, 10, 1,000	6	3	50	4.5
Fresh serum with sulfapyridine	1, 10, 1,000	6	6	100	7
Heated serum at 56° C. alone*	1, 10, 1,000	6	0	0	2
Heated serum at 56° C. with sulfapyridine	1, 10, 1,000	6	4	83	5.3
Sulfapyridine alone	1, 10, 1,000	6	0	0	2.5
Culture controls	1, 10, 1,000	6	0	0	1.8

* Heated for 30 minutes.

adult serum (24 hours after bleeding) gave 50 per cent protection, but the same serum heated for 30 minutes at 56° C. gave no protection when used without sulfapyridine. The heated serum, when given with sulfapyridine, protected the mice to a lesser extent than the unheated, fresh serum given with sulfapyridine. One other serum tested, fresh and heated, gave similar results.

None of the absorbed sera gave any protection when absorbed with homologous cultures, whether used alone or with sulfapyridine. Absorption with heterologous cultures removed most of the protective substances, but not all, since some protection still resulted from these sera, even when very large doses of cultures were used for absorption. Absorption with kaolin had no effect on the sera. Nor did the absorbed sera, when injected without culture, have any effect on the mice. Table III represents a test with absorbed sera.

Protection against pneumococcus Type III

No direct parallelism was noted in the action of the sera when tested against Type I and Type III pneumococci. Whereas the great majority of

TABLE III

Mouse protection test with normal human adult serum (757A) absorbed with Type I and Type II pneumococcus cells against Type I and Type II pneumococcus cultures

Material injected	1, 10, 1,000 M.L.D.			
	Type I culture		Type II culture	
	Number recovered	Number died	Number recovered	Number died
Serum absorbed with Type I cells alone	0	6	0	6
Serum absorbed with Type I cells with sulfapyridine	0	6	2	4
Serum absorbed with Type II cells alone	0	6	0	6
Serum absorbed with Type II cells with sulfapyridine	1	5	0	6
Unabsorbed serum alone	3	3	3	3
Unabsorbed serum with sulfapyridine	6	0	5	1
Sulfapyridine alone	0	6	0	6
Culture controls	0	6	0	6

the normal adult human sera did not show any demonstrable protective ability against Type I organisms when used alone, and showed a definite increase in survival rate when used with sulfapyridine, such sera protected mice against Type III strains and failed to increase the survival rate of

TABLE IV

Mouse protection test with normal human adult serum (AA107) used fresh and heated with and without sulfapyridine against Type III pneumococcus

Treatment	Number of M.L.D. of culture	Number of mice infected	Number of mice recovered	Per cent recovered	Average days survived
Fresh serum alone	1, 10, 100, 1,000, 10,000, 100,000	12	1	8.4	2.6
Fresh serum with sulfapyridine	1, 10, 100, 1,000, 10,000, 100,000	12	3	25	4.7
Heated serum at 56° C. alone*	1, 10, 100, 1,000, 10,000, 100,000	12	2	16.6	2.8
Heated serum at 56° C. with sulfapyridine	1, 10, 100, 1,000, 10,000, 100,000	12	6	50	5.7
Heated serum at 60° C. alone†	1, 10, 100, 1,000, 10,000, 100,000	12	4	33.3	3.5
Heated serum at 60° C. with sulfapyridine	1, 10, 100, 1,000, 10,000, 100,000	12	8	66.6	5.8
Sulfapyridine alone	1, 10, 100, 1,000, 10,000, 100,000	12	10	83	6.6
Culture controls	1, 10, 100	6	0	0	2

* Heated for 30 minutes.

† Heated for one hour.

the sulfapyridine-treated mice, producing instead a decrease in the protective action of the sulfapyridine. There was a definite inhibition in the protective activity when the sera were used with Type III organisms. This inhibitory action was decreased but not entirely destroyed by heating. Table IV represents a typical experiment showing this inhibitory action.

Protection against other types

One normal human adult serum was tested against Types II, IV, V, VI, IX and XVIII, with and without sulfapyridine. It gave 100 per cent protection both when used alone and with sulfapyridine, against 1,000 M.L.D.'s of virulent organisms. Since no end point of protection was obtained with the amounts of culture used, it was impossible to determine whether the serum with sulfapyridine increased the survival rate, when tested against these types.

DISCUSSION

Although pneumococcal antibodies are encountered in the blood of normal human adults with great frequency (1 to 11), mouse protective activity of normal human sera is usually found to be low, or not demonstrable at all (8). With the aid of sulfapyridine, used in amounts so small that by itself it did not give any protection, we were able to demonstrate protective antibodies against Type I pneumococci in all the normal human adult sera tested, although 10 of the 12 sera examined did not show any protection when used alone. On the other hand, when sera from normal human infants, aged 4½ to 7 months, were examined, no protection was apparent, whether the sera were used alone or with sulfapyridine. These results are in agreement with the work of others (12 to 14), who found that the pneumococcal antibodies in very young infants, obtained through the passive transfer from the mother, are exhausted at the end of 5 to 6 weeks, and reappear in the blood of the child when about a year old.

Robertson and Sia (11) as well as others (9, 15 to 17) found that the common laboratory animals, exhibiting a high degree of resistance to pneumococci, such as the horse, sheep, dog, cat

EFFECTS OF BARBITURATE ANESTHESIA (EVIPAL AND PENTOTHAL SODIUM)
UPON THE INTEGRATION OF RESPIRATORY CONTROL MECHANISMS.
A STUDY DIRECTED TOWARD IMPROVEMENT OF METHODS
FOR THE PRECLINICAL EVALUATION OF
ANESTHETIC AGENTS

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General Hospital, Boston)

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In previous articles the effects of sodium evipal² or sodium pentothal³ anesthesia upon the respiratory control mechanisms were described. Both anesthetics depress the central chemical mechanism of breathing (carbon dioxide control) much more rapidly than they depress the peripheral reflex chemical mechanisms (carotid and aortic chemoreflexes) (1), and the vagopulmonary proprioceptive reflexes (Hering-Breuer) (2). These observations are in agreement with the earlier findings of Henderson (3), Marshall and Rosenfeld (4), and Comroe and Schmidt (5) for anesthetic states produced by other barbiturates.

The experiments reported here were planned so that the relative rôles played by carbon dioxide and by oxygen, in the control and maintenance of breathing at different depths of evipal and pentothal anesthesia, could be more fully evaluated. Accordingly, the respiratory responses to a constant increase in the carbon dioxide content, and to a constant decrease in the oxygen content of the inspired air were recorded at three levels of anesthesia. This provided a gross measure of the ability of an animal (dog) to react to fairly constant chemical stimulation of the respiration. The stimulating agents chosen exhibit certain differences in their sites of action. In addition, the effects of increasing the oxygen tension of arterial blood above that present when the animals breathed room air were studied in order to determine roughly the degree of anoxic chemoreflex activity at different anesthesia levels when the animals were breathing air.

It was learned from earlier experiments that as the anesthesia (evipal or pentothal⁴) was deepened, breathing became more dependent upon anoxic chemoreflex stimulation without, in many instances, any great change in minute volume of breathing; therefore, it was realized that it was necessary to keep the oxygenation of arterial blood at or above that existing at the beginning of the experiment in order to appreciate even roughly the rate and extent to which the mechanisms involved in hypercapnic hyperpnea were depressed. In order to accomplish this, the anesthesia was increased while the animals breathed pure oxygen. The efficacy of this procedure was checked by frequent determinations of the oxygen content of the arterial blood. The possibility existed that the decreased breathing that normally accompanied deepening anesthesia might have been due to a decreasing rate of carbon dioxide production, as a result of a reduction in the normal oxidations effected by the anesthetic, rather than to a decreased reactivity of the animal to carbon dioxide. To check this possibility, the carbon dioxide content of the arterial blood was determined at close intervals.

These things were done with the hope that it might thereby be possible to establish certain improved basic methods for evaluating new anesthetic agents before their clinical introduction. Final evaluation of anesthetic agents can be made, of course, only in the clinic, but the wastefulness of present practices, not only in monetary terms but in terms of human lives needlessly lost, offers a stimulating challenge to improve laboratory methods of evaluating anesthetic agents before they are introduced into the clinic.

⁴ Whenever evipal or pentothal are administered, it is to be understood that reference is made to the soluble sodium salt of the agent.

¹ Fellow of the National Research Council.

² Sodium evipal is 1-methyl 5Δ¹ cyclohexenyl 5 methyl barbiturate.

³ Sodium pentothal is ethyl (1-methyl-butyl) thiobarbiturate.

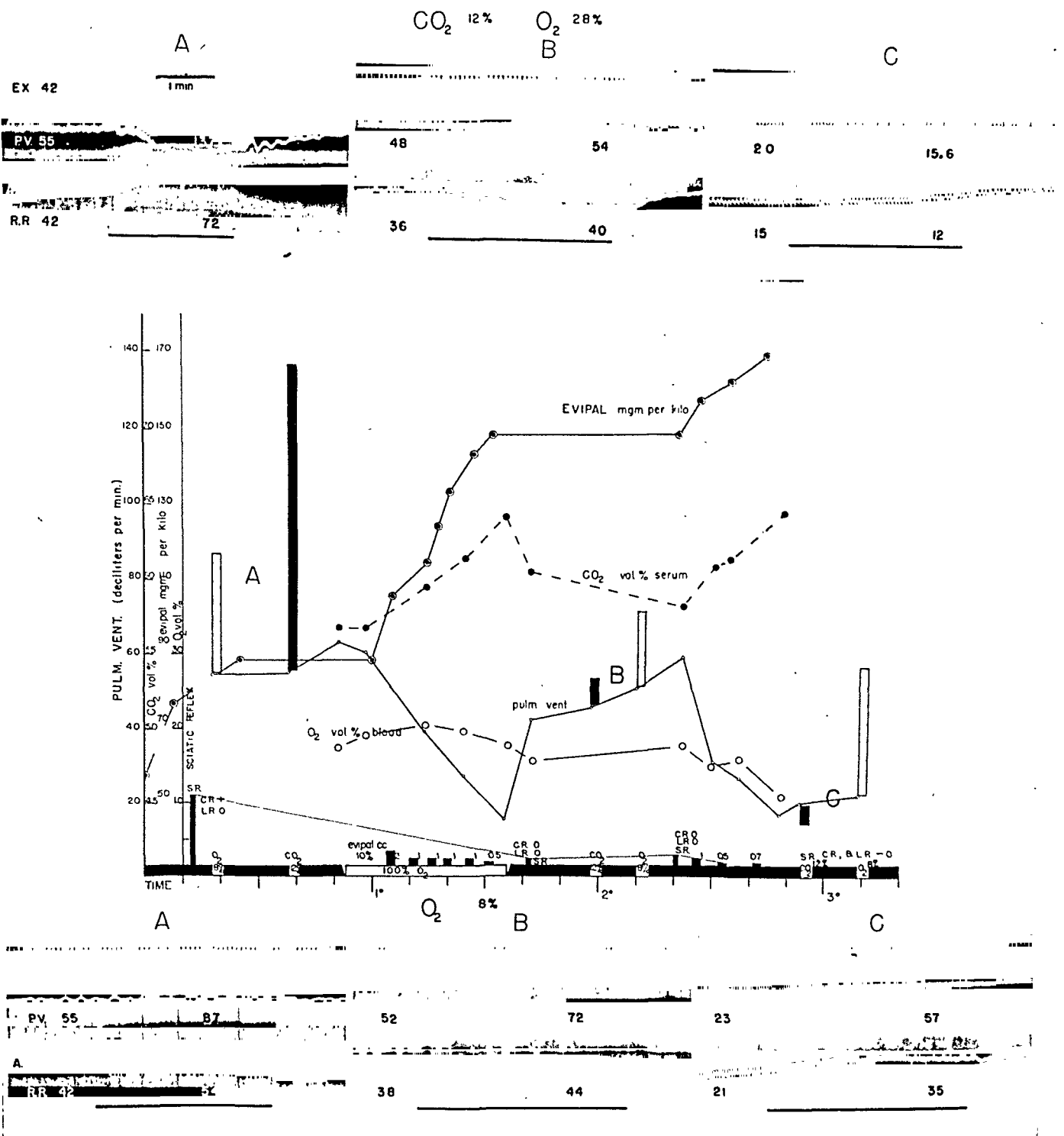


FIG. 1. TIME RELATIONSHIPS OF THE FACTORS CONSIDERED

The kymographic tracings show, from above downward, time in 5 second intervals, costal respiration, the pulmonary minute ventilation (P.V.) in deci-liters (the spirometer tracings of the tidal excursions, used in the calculation of P.V., have been omitted to conserve space), abdominal respiration, blood pressure (at varying positions on the several records, easily differentiated from the respiratory tracings by its smaller excursion and characteristic appearance), respiratory rate per minute (R.R.), a fine base line (used in some subsequent figures to indicate activity of the sciatic reflex, as an objective record of anesthesia depth), a broad line to indicate the interval during which special gas mixtures were administered. The special gas mixtures are indicated above the respective kymograph tracings; it is to be understood that nitrogen adequate to complete the 100 per cent is included with the specified gases.

The chart shows five ordinate scales, from left to right: pulmonary ventilation in deci-liters per minute, carbon dioxide in volumes per cent of arterial serum, dose of evipal in mgm. per kgm. body weight, oxygen in vol-

METHODS

The equipment and procedures employed have been described previously (1). Briefly, mongrel dogs weighing about 10 kgm. were used as subjects. Data pertinent to the present report were obtained from experiments on 29 dogs; but since these first 29 experiments were directed primarily toward a solution of other although related problems, 8 more dogs were used in which the specific questions raised in this paper were studied. The anesthetic agents studied consisted of *sodium evipal* [1 methyl 5 α cyclohexenyl 5 methyl barbiturate] in 10 per cent aqueous solution, administered intravenously, and *sodium pentothal* [ethyl (1 methyl-butyl) thiobarbiturate] in 5 per cent aqueous solution also administered intravenously. Depth of anesthesia was controlled as carefully as possible by observation of records of a flexion reflex (left semitendinosus muscle) elicited by electrical stimulation of the central end of the cut left sciatic nerve. In addition, notes were kept of the state of the corneal (C.R.) and lid (L.R.) reflexes; normal activity was described as 2+. Three simultaneous records were made of the respiration: tidal, "intercostal," and "diaphragmatic." Arterial blood pressure was recorded by means of a Ludwig manometer connected with the femoral artery. Blood gases were determined in duplicate by the methods of Van Slyke and Neill; oxygen in 1.0 cc. samples of heparinized arterial whole blood taken under oil, carbon dioxide in 0.5 cc. of arterial serum, collected under oil.

PRESENTATION OF RESULTS

The kymographic tracings show from above downward: time in 5 second intervals, costal breathing (up-stroke-inspiration), respiratory minute volume in deciliters per minute (the spirometer tracings from which the values of pulmonary ventilation or minute volume were obtained have been omitted to conserve space in all the figures excepting number 9), abdominal respiration, respiratory rate (R.R.), blood pressure (at varying positions on the several records, easily differentiated from the respiratory tracing by its smaller excursion and characteristic appearance), the base line of activity of the sciatic reflex (see Figure 9), a broad white inked line to indicate the interval during which special gas mixtures were administered, and blood pressure base line (all the records excepting that in Figure 9 were cut along the corrected base line for blood pressure). The special gas mixtures (CO₂, 12 per cent and O₂, 28 per cent, and O₂, 8 per cent) are indicated above the respective kymo-

graph tracings; it is to be understood that nitrogen completed the 100 per cent.

The graphs in the illustrations (1 to 8 inclusive) show the time relationships of all the procedures of the experiments. The ordinate scales are five in number and are from left to right as follows: pulmonary ventilation (respiratory minute volume) in deciliters per minute, carbon dioxide in volumes per cent of arterial serum, the additive amount of evipal or pentothal in mgm. per kgm. of body weight (exact placement of this ordinate varies in different figures), oxygen in volumes per cent of arterial blood, and height of the reflexly excited contraction of the semitendinosus muscle in mm.

The abscissa shows: the time relationships of the experiments (the unit interval represents 10 minutes); the gas mixture breathed (solid black designates the periods when room air is respired, the short unroofed open blocks labeled O₂, 8 per cent and CO₂, 12 per cent delimit the periods of the low oxygen or carbon dioxide administrations, and the completely enclosed clear blocks represent the periods when oxygen was breathed); and the number of cc. of evipal (10 per cent) or pentothal (5 per cent) injected.

The solid bar graphs (S.R.) based on the abscissa denote the heights of contraction of the semitendinosus muscle. The fine line that connects the tops of these bars grossly indicates the trend of anesthesia, a falling line indicates greater depth.

The bar graphs based on the line designated "pulm. vent." represent when solid, carbon dioxide hyperpnea, and when cross hatched, low oxygen hyperpnea, measured as the increase in minute volume of breathing obtaining at the end of the third minute of the carbon dioxide or low oxygen administration, over the normal minute volume of breathing for the last three minutes of eupnea, excepting in experiment 43, Figure 4, in which the normal was taken as the minute volume of breathing, during the seventh minute after the carbon dioxide administration was terminated because of the failure of the animal to return to the apneustic type of respiration that prevailed before the period of hypercapnia. The records, excepting the spirometer tracings from which the data for these later bar graphs were obtained, are shown above (carbon dioxide) and below (low oxygen) their graph placements; these records do not coincide at the positions A, B, or C but lie on either side of them. The data, from which the line graph labeled "pulm. vent." was constructed, was obtained from the spirometer record of tracings taken of breathing after a grossly steady rhythm had been attained and before changes in experimental condi-

tures per cent of arterial whole blood. The sciatic reflex activity is indicated in mm. contraction of the semitendinosus muscle. The state of the corneal (C.R.) and lid (L.R.) reflexes is shown.

The abscissa is used to show time relationships, the intervals (and dose) at which the anesthetic agent was administered, and the particular gas mixtures inspired. Room air was inspired except as specified.

The bar graphs in the body of the figure indicate when solid, carbon dioxide hyperpnea (refer to pulmonary ventilation ordinate); when cross-hatched, low oxygen hyperpnea; the height of each of these indicates the increase over "normal" is a result of the particular stimulant. The letters A, B, C, identify on the graph the position of the kymographic data shown.

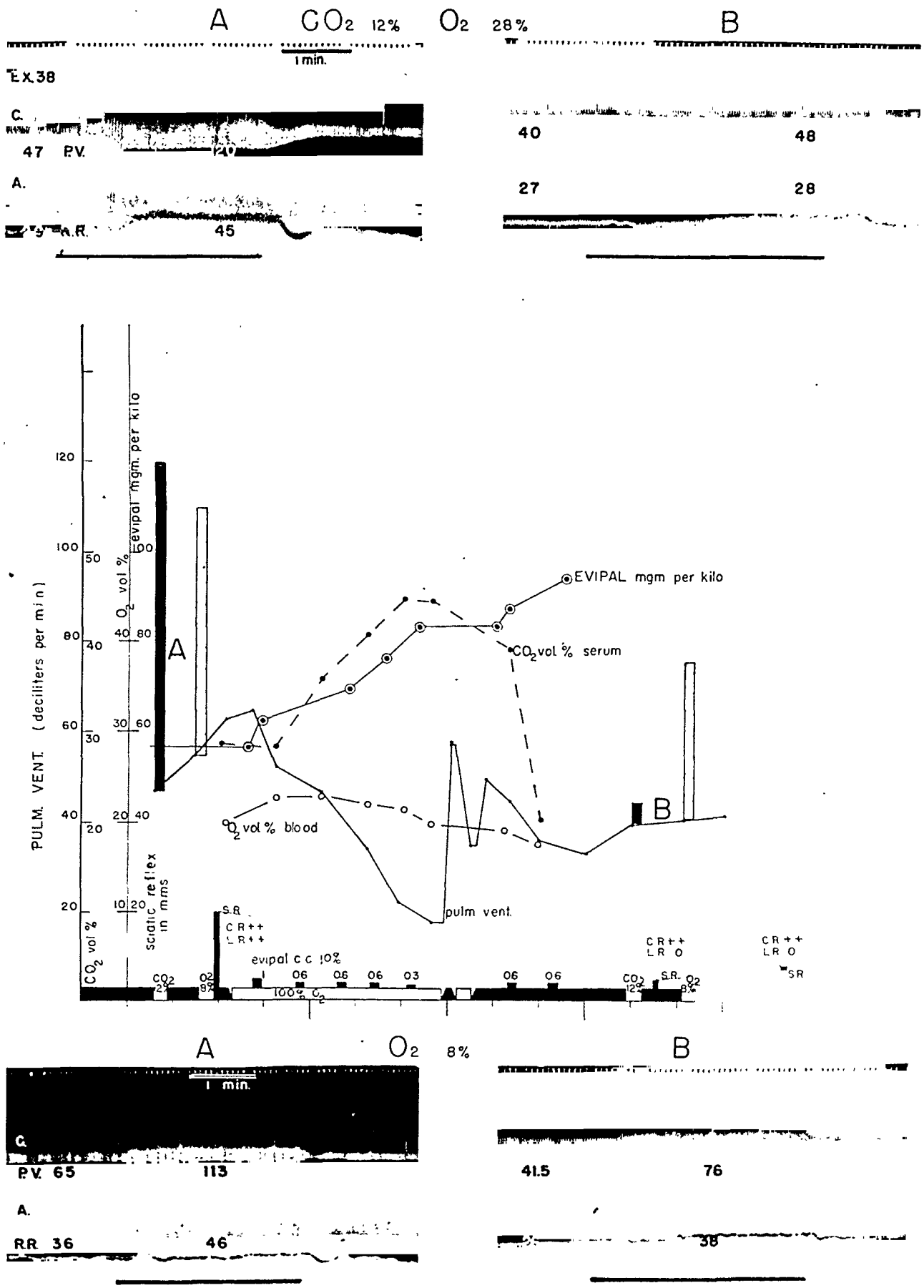


FIG. 2. LEGEND AS FOR FIGURE 1

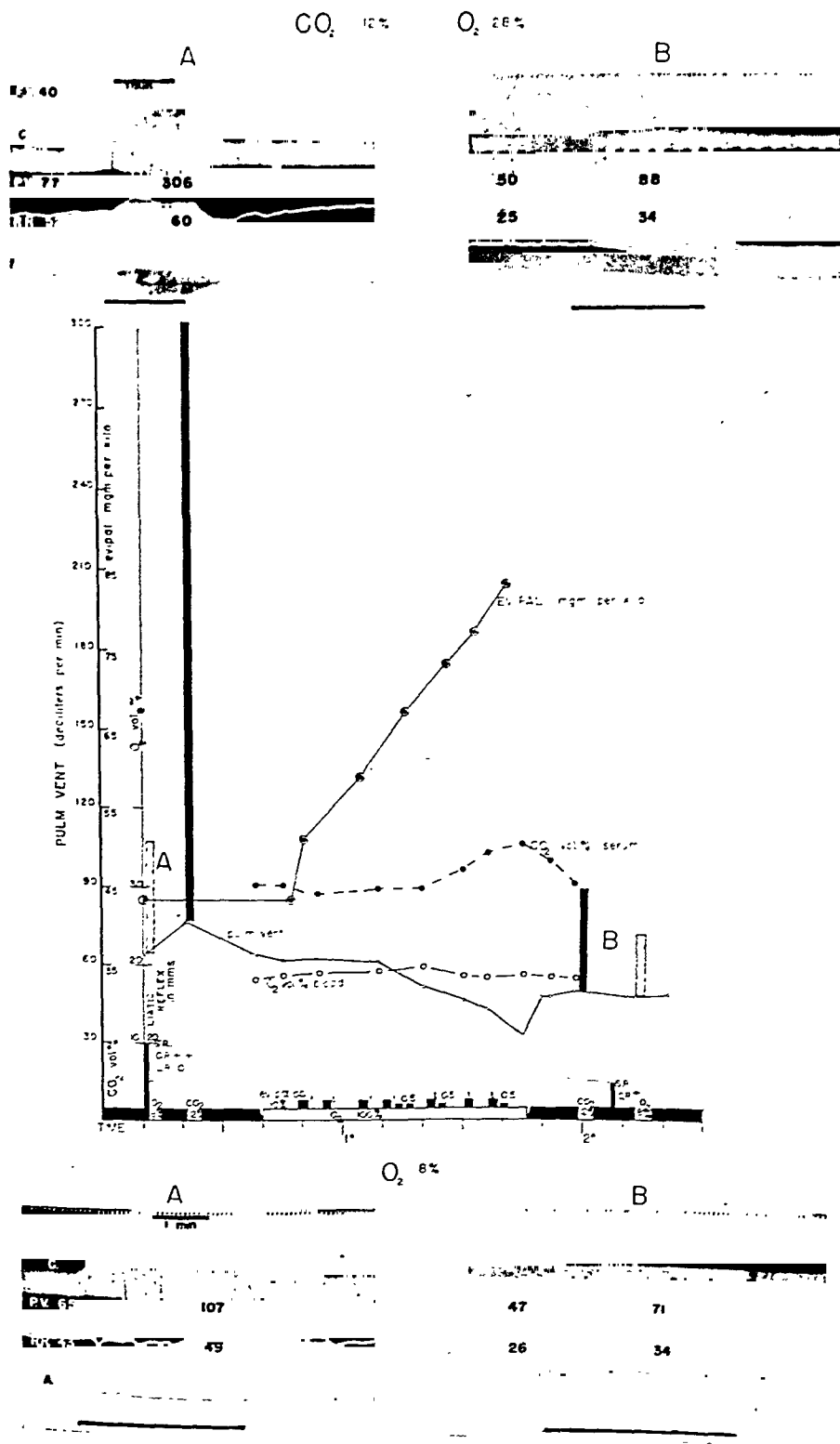


FIG. 3. LEGEND AS FOR FIGURE 1



tions were made (the administration of oxygen or air, the injection of anesthetic, or the withdrawal of arterial blood samples, etc.). It is evident that evipal (Figures 1 to 4) and pentothal (Figures 5 to 8) exert a much greater depressant effect on hypercapnic hyperpnea than upon anoxic hyperpnea. In three instances (Figures 1, 4, and 6) carbon dioxide changed from a powerful respiratory stimulant to a depressant. The depressant action does not require that a high degree of anoxemia pre-exist; the oxygen content of the arterial blood in one instance was normal just before the depressant effect of carbon dioxide was elicited (Figure 4, experiment 43).

In order to obtain clearer information as to the rapidity with which central sensitivity to carbon dioxide was reduced as previously mentioned, evipal and pentothal were given while the animals breathed 100 per cent oxygen in order to keep the chemosensitive reflex mechanisms as inactive as possible. The anesthesia was always very light when this change was first effected. Arterial blood oxygen content rose 0.4 to 3.3 volumes per cent, but arterial carbon dioxide did not change significantly when 100 per cent oxygen was given (Table I). It rose, however, as more anesthetic was given. With each injection of evipal or pentothal while the animal breathed oxygen, the minute

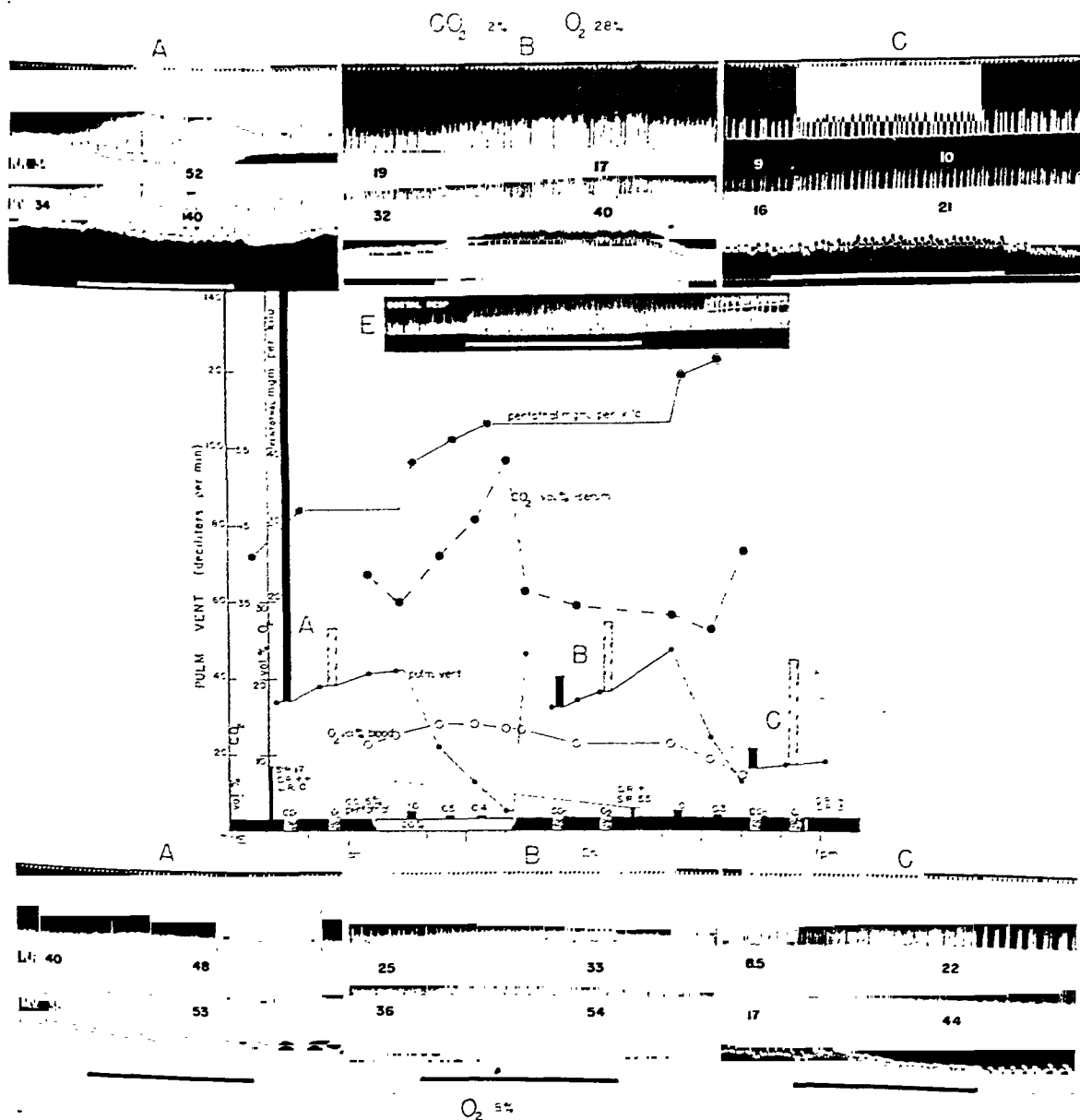


FIG. 5. LEGEND AS FOR FIGURE 1

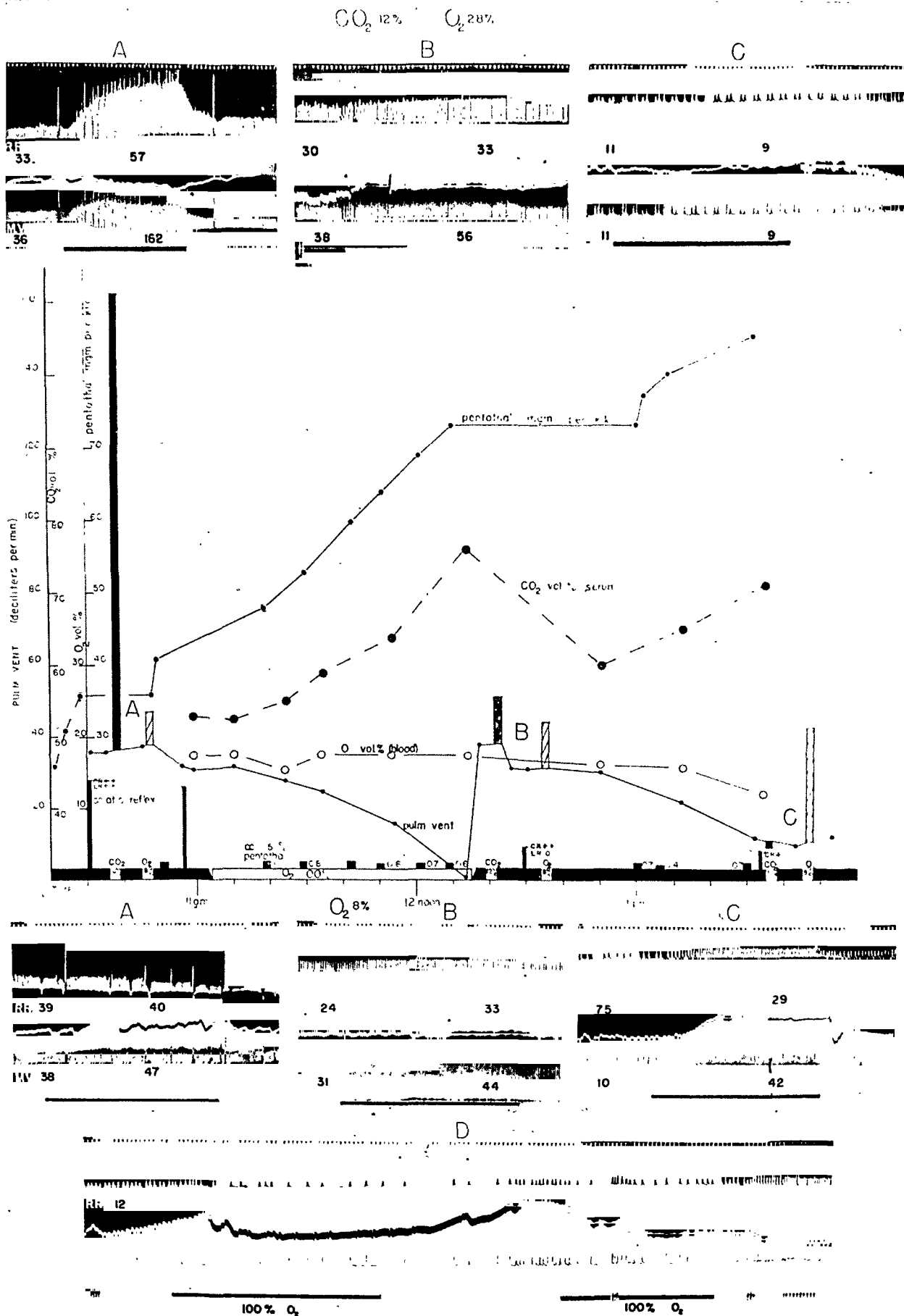


FIG. 6. LEGEND AS FOR FIGURE 1

volume of breathing fell, mainly as a result of a slowing of rate. (The evipal and pentothal injections are shown in bars along the abscissa; the additive quantity of drug in mgm. per kgm. is represented as a line graph.) The individual variation to evipal or pentothal, as determined by the variability between the decreases in minute volume of breathing per mgm. of drug per kgm., is great even in these very small series. Compare Figures 1 with 2, and 5 with 8.

With the decrease in minute volume of breathing, serum carbon dioxide rose indicating that breathing was depressed by evipal and pentothal more rapidly than was the rate of carbon dioxide formation, if indeed the latter was at all affected at the anesthesia level concerned. The expedient of administering 100 per cent oxygen to keep the level of anoxic chemoreflex respiratory stimulation at or below normal limits was fairly successful, for in only two experiments (46 and 52) did the arterial oxygen con-

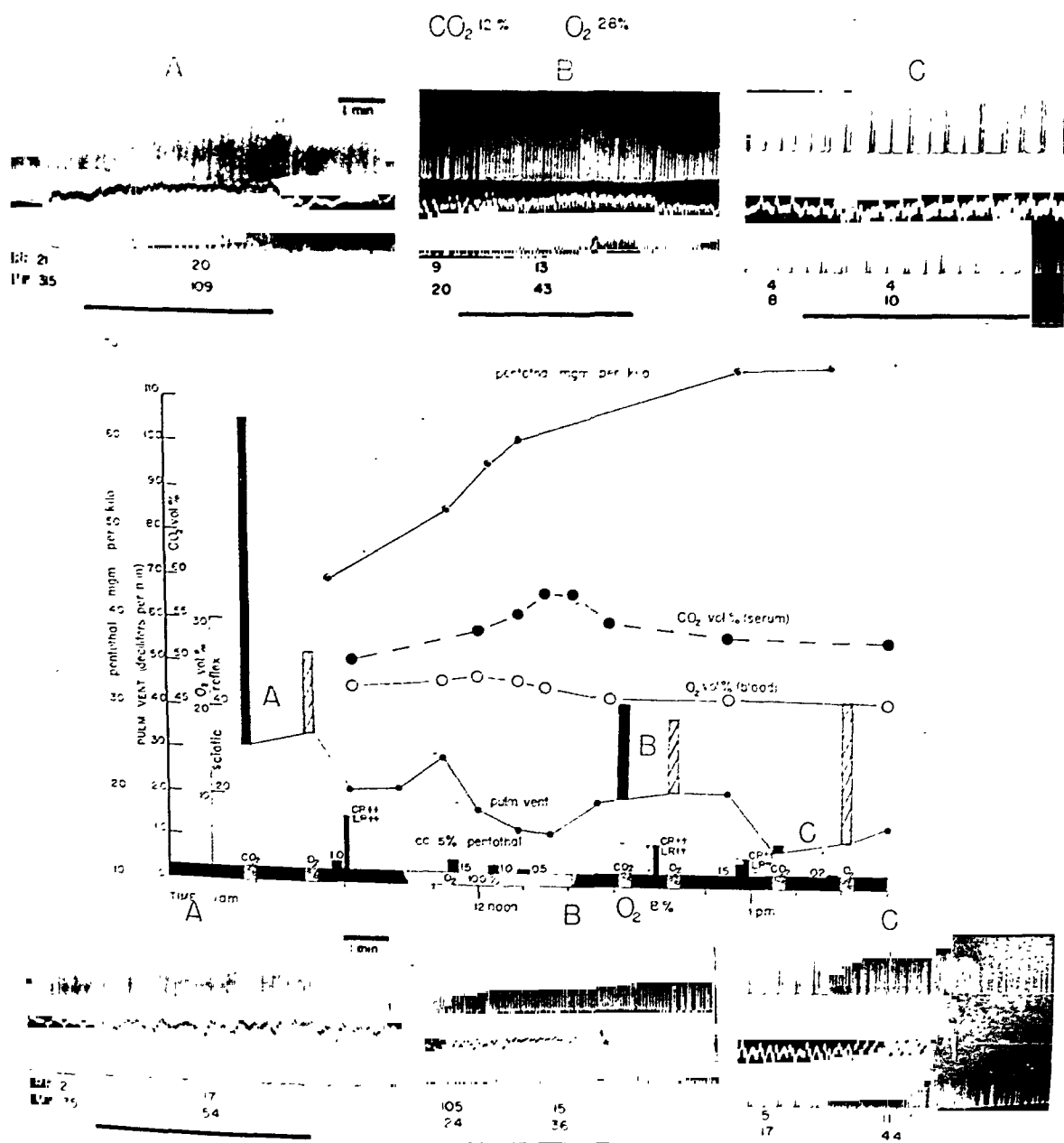


FIG. 7. LEGEND AS FOR FIGURE 1

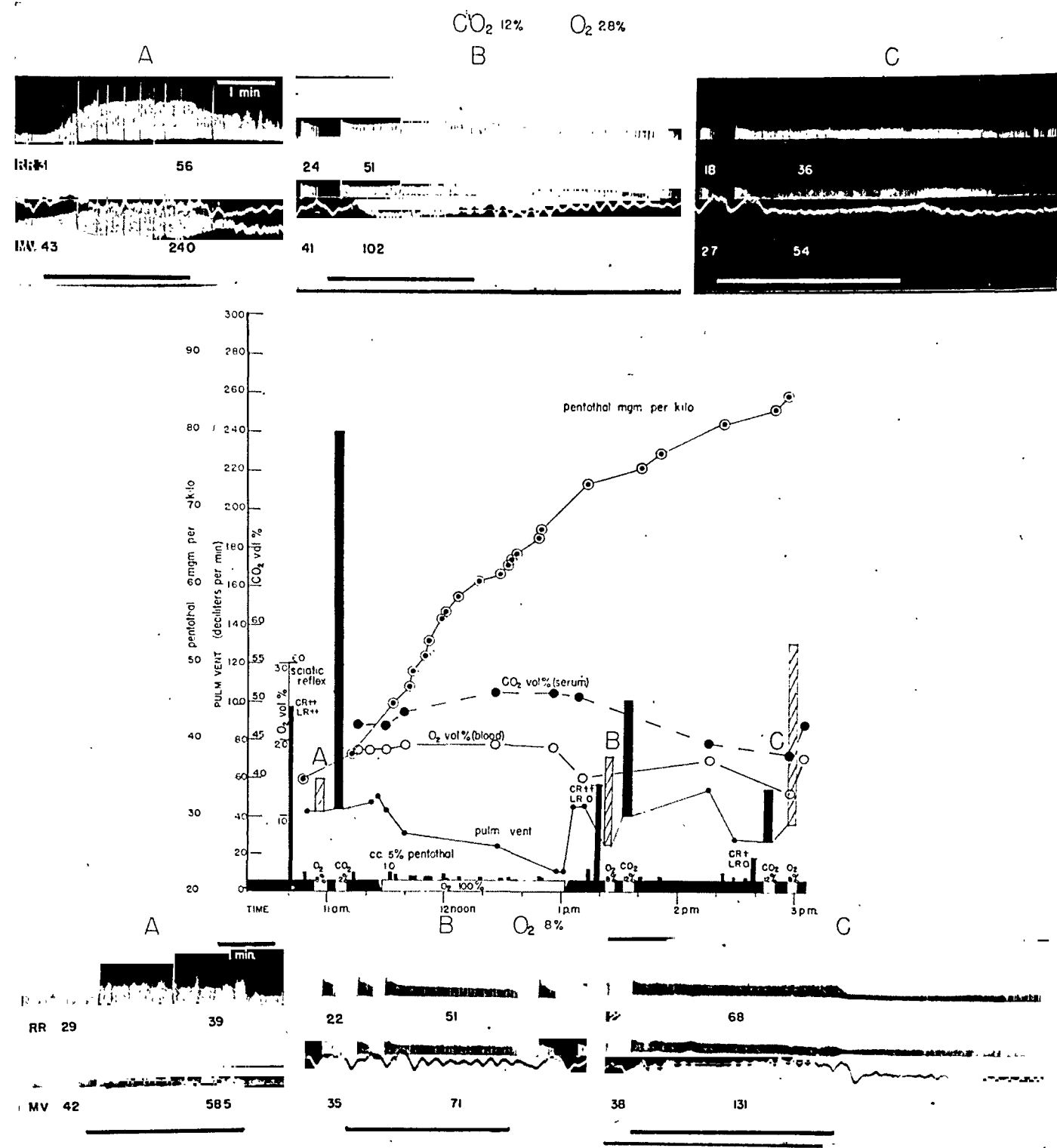


FIG. 8. LEGEND AS FOR FIGURE 1

tents fall below the control value, in spite of reductions in minute volume of breathing. When room air was again administered, breathing quickly increased. The increases in minute volume of breathing were associated with decreases in arterial carbon dioxide and oxygen contents (Table I).

DISCUSSION

It appears, from the evidence gained from these experiments, that the breathing of an ani-

mal very lightly anesthetized with evipal or pento-
thal is primarily regulated by the need for carbon
dioxide removal and not especially by need for
oxygen, which agrees with the findings of Haldane
(6) and others in normal man and animals. If
respiration was being regulated substantially by
oxygen need, then slowing of breathing should
have been the rule when the arterial oxygen ten-
sion was increased. The oxygen content of arte-

TABLE 1
Arterial O₂ volumes per cent

Experiment number	Anesthesia Light		Anesthesia Moderate		Anesthesia Deep	
		1st. detem. during 100 per cent O ₂	Last detem. during 100 per cent O ₂	1st. detem. after 100 per cent O ₂	Last detem. air	Minute volume increase (liters) when shifted from 100 per cent O ₂ to air
Evipal						
38	20.0	23.3	20.0	17.7		2.7 in 6 minutes
40	16.9	17.5	17.0	17.0		1.3 in 4 minutes
42	17.5	19.3	17.9	15.7	15.5	2.7 in 2 minutes
43	19.0	20.0	20.4	19.3	17.3	2.1 in 3 minutes
Pentothal						
44	12.7	14.0	13.4	13.2	7.1	4.2 in 4 minutes
46	17.1	17.8	16.4	15.8	12.0	3.5 in 3 minutes
52	22.0	22.5	21.5	21.0	20.0	3.3 in 2 minutes
53	17.8	18.2	17.8	15.4	17.2	0.7 in 7 minutes
	Air	Oxygen—100 per cent		Air		(time between shift and time maximum MV was reached)

Arterial CO₂ volumes per cent

Experiment number	Anesthesia Light		Anesthesia Moderate		Anesthesia Deep	
		1st. detem. during 100 per cent O ₂	Last detem. during 100 per cent O ₂	1st. detem. after 100 per cent O ₂	Last detem. air	Minute volume increase (liters) when shifted from 100 per cent O ₂ to air
Evipal						
38	25.9	25.5	44.8	39.4		
40	45.7	45.5	50.2	45.4		
42	58.8	58.2	73.8	66.4	74.3	
43	56.8	57.1	72.1	65.3	73.4	
Pentothal						
44	37.0	34.9	53.0	36.1	42.4	
46	53.0	52.6	76.4	60.2	71.3	
52	50.0	54.0	58.0	51.5	53.0	
53	46.5	47.8	51.0	50.0	48.0	
	Air	Oxygen—100 per cent		Air		

rial blood increased 0.4 to 3.3 volumes per cent and the carbon dioxide content of the arterial serum did not change in these experiments under light anesthesia when 100 per cent oxygen was substituted for room air; in only 2 of the 8 experiments was there a significant sustained decrease in rate or amplitude of breathing associated with this increased oxygenation. Pertinent supporting data can be found in the observation that minute volume of respiration necessary for the maintenance of a normal arterial oxygen content of some anesthetized animals at sea level is less than that necessary to effect removal of carbon dioxide. This becomes evident in these studies only after the respiratory center's sensitivity to carbon dioxide is reduced, as in experiment 43, Figure 4. When the animal after being more deeply anesthetized is breathing room air, an arterial oxygen content is maintained at the normal level by one-third the minute volume that was present when the animal was lightly anesthetized, but the arterial carbon dioxide continues to increase at this lowered level of ventilation.

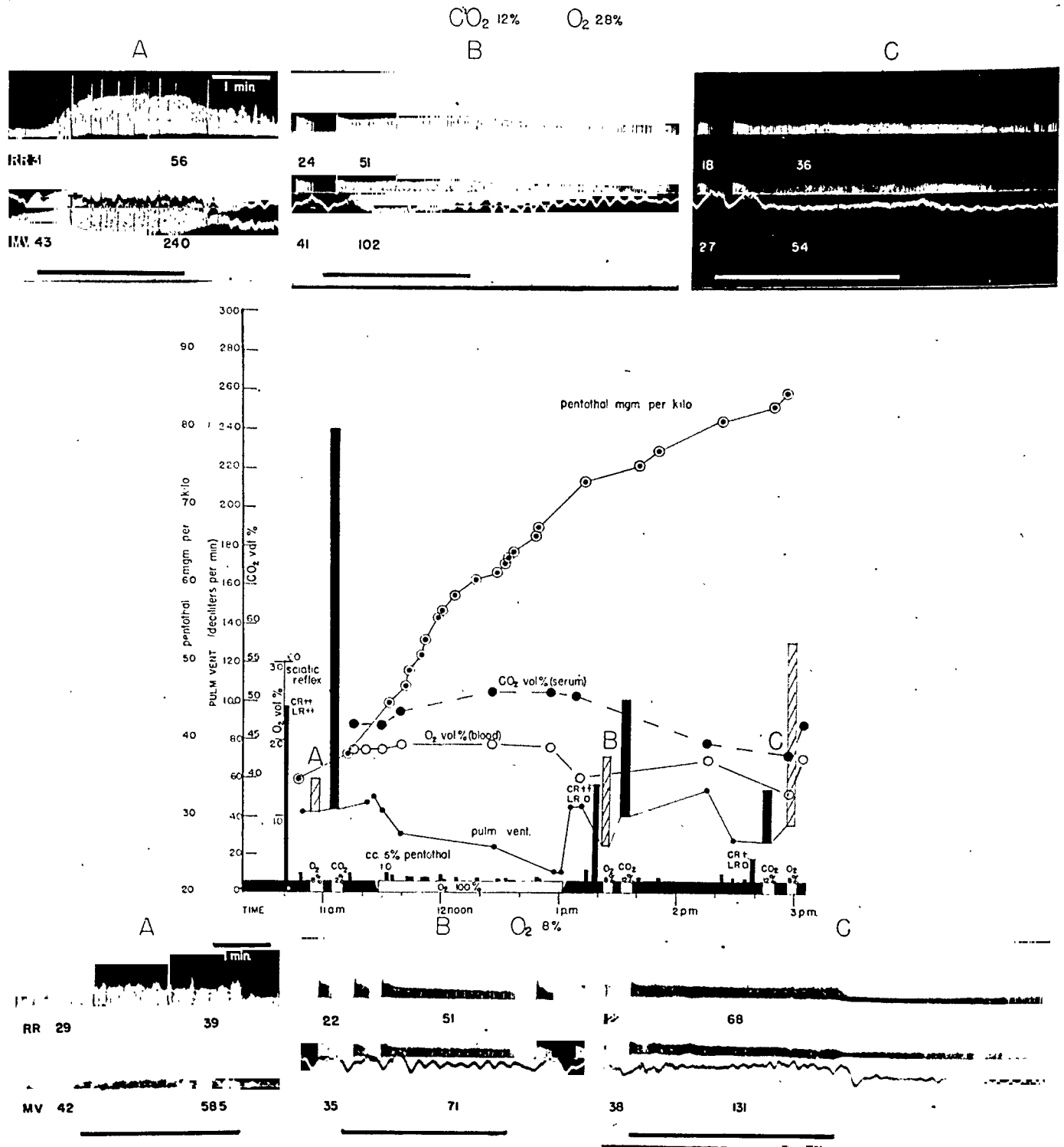
As the evipal anesthesia is moderately deepened, breathing is seemingly regulated by both carbon dioxide and oxygen need. At this stage

100 per cent oxygen visibly slows breathing and carbon dioxide is still able to stimulate it. The intermediate stages (B) of experiments 1 to 8 illustrate this point. Carbon dioxide inhalations still stimulate breathing appreciably and the decrease in arterial oxygen content of from 0.2 to 2.4 volumes per cent that follows the shift from 100 per cent oxygen to room air is associated with minute volume increases varying between 0.7 to 4.2 liters per minute. If pure oxygen is administered again, the pulmonary exchange decreases [Figures 2 (above second clear block of abscissa) and 4 (lower right kymographic record)].

When the anesthesia is deepened, further breathing becomes increasingly dependent upon arterial oxygen content, since carbon dioxide becomes less capable of stimulating breathing and instead occasionally depresses it (Figures 1, 4, and 6). Increase in the oxygen content of the arterial blood now slows respiration dangerously. This is shown in Figures 4 (lower left) and 6.

Mechanisms evidently playing the major rôle in maintenance of breathing, after central sensitivity to carbon dioxide is obliterated, are the carotid and aortic chemoreceptors (4, 7, 8). At this stage of anesthesia it is probable that the chemoreceptor mechanisms have a lower threshold to oxygen lack stimulation than does the center, which is in complete accord with the findings of Heymans (9) and others. *This evident shift in maintenance of eupneic breathing, from the action of carbon dioxide upon the automatically rhythmic center to control by the chemosensitive reflex mechanisms activated by anoxia, is in many instances unattended by changes in the rate or character of breathing, or changes in blood pressure, which might afford us objective means of determining when the shift takes place. Therefore, the anesthetist has no means of knowing the status of the respiratory control mechanisms when employing evipal or pentothal.*

These experiments permit some insight into possible interrelations of the unanesthetized center and chemoreceptors in the regulation of breathing during hypoxia. In 6 of the experiments illustrated here, the stimulation of breathing by a constant degree of hypoxia increased as the anesthesia was deepened. This takes place occasionally when evipal or pentothal is injected during the hypoxic hyperpnea (3 of 10 experiments with evipal and



tents fall below the control value, in spite of reductions in minute volume of breathing. When room air was again administered, breathing quickly increased. The increases in minute volume of breathing were associated with decreases in arterial carbon dioxide and oxygen contents (Table I).

DISCUSSION

It appears, from the evidence gained from these experiments, that the breathing of an ani-

mal very lightly anesthetized with evipal or pentothal is primarily regulated by the need for carbon dioxide removal and not especially by need for oxygen, which agrees with the findings of Haldane (6) and others in normal man and animals. If respiration was being regulated substantially by oxygen need, then slowing of breathing should have been the rule when the arterial oxygen tension was increased. The oxygen content of arte-

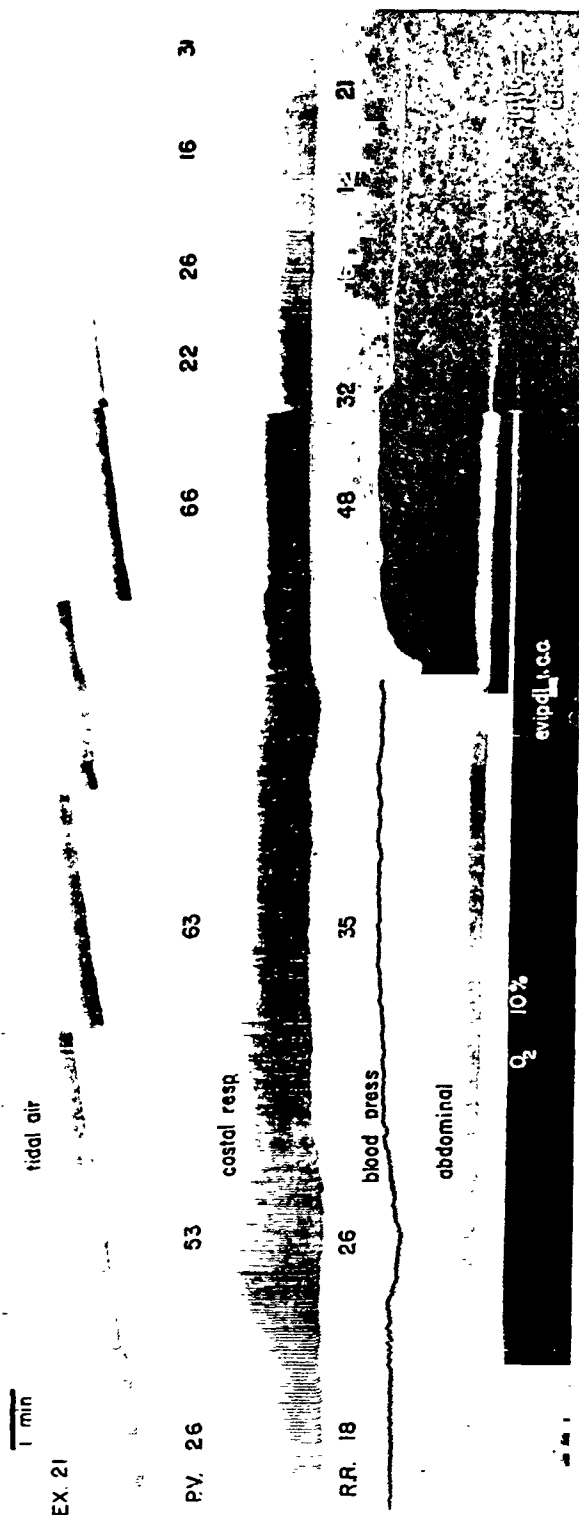


FIG. 9. INCREASED EFFECTIVENESS OF HYPOXIA AS A RESPIRATORY STIMULANT WITH INCREASED DEPTH OF ANESTHESIA

The kymographic tracings show from above downward: time in 5 second intervals, tidal air, pulmonary ventilation (P.V.) in deciliters, costal respiration, respiratory rate per minute (R.R.), arterial blood pressure, abdominal respiration, interval (broad band) during which 10 per cent oxygen was administered (otherwise room air), sciatic reflex (record of contraction of the semitendinosus muscle), the interval during which 1.0 cc. 10 per cent evipal was administered, and the state of the corneal reflex (C.R.).

oxide content of the arterial blood, as they are to effecting adjustments to decreased oxygen contents and tensions. This does not imply that the carbon dioxide is not stimulating the glomus endings, for Samaan and Stella (12) and von Euler, Liljestrand, and Zotterman (13) have shown increasing discharges in Hering's nerve as carbon dioxide is increased in the arterial blood. It is possible that carbon dioxide blocks the propagation of the impulses by its reflex depressant action (14, 15, 16).

The significant changes in the control of breathing that attend small variations in anesthetic states induced by evipal or pentothal are not apparent from the determination of the relationship of dosage to respiratory minute volume of breathing while the animals breathe air. The usual methods of determining whether an anesthetic is of probable clinical value leave too much to be learned concerning its pharmacologic action during its clinical employment with a relatively large expenditure of effort, time, and human life. This is apparently in part dependent upon the difficulty of anticipating, ahead of clinical use, the multiplicity and extent of the demands placed upon the agent by the surgeon and the anesthetist, and to general lack of recognition of the extent to which alterations in physiologic state incident to disease, medication, and surgery may affect the toxicity of the anesthetic. Lives might well have been saved had it been known when evipal and pentothal were introduced into the clinic, that hypercarbia and anoxia increase the toxicity of these agents (1) and that low blood oxygen can mask the true level of anesthesia (1). Hyperoxia (oxygen content of inspired gas greater than that of air), for reasons previously discussed, increases the respiratory toxicity of evipal and pentothal. In Figures 1 to 8 inclusive it is clear that less barbiturate is needed to effect a given decrease in minute volume of breathing when oxygen is breathed than when air is breathed. This was not known when the anesthetist first used evipal and pentothal and the fear of the anoxia, that was indicated by the frequent appearance of cyanosis during the course of this anesthesia, rightly prompted the administration of high oxygen concentrations in the inspired air. However, the breathing of oxygen during evipal

and pentothal anesthesia, as a consequence of the reduction of the sensitivity of the respiratory center to carbon dioxide by the drugs, coupled with the reduction of the chemoreflex stimuli by the increased oxygenation of arterial blood, results in hypopnea with a retention of carbon dioxide by the body as indicated by the rise in the carbon dioxide content of the arterial blood (Table I). During the period of oxygen administration the arterial oxygen content was either at or slightly above the normal and cyanosis consequently never developed (Table I). Therefore, oxidations, as far as an anesthetist could determine, were never jeopardized; however, the retention of carbon dioxide that occurred without an external sign may well have depressed cellular oxidations more than the direct oxygen lack that may have occurred if room air had been breathed. Furthermore, the retention of carbon dioxide during the oxygen administration probably increases the toxicity of evipal and pentothal more than would the usually minor degree of anoxia that is incurred when room air is breathed. It is, therefore, apparent that the adoption of a seemingly simple, effective, and innocuous procedure by the anesthetist, without knowing its effect upon the homeostasis of an organism under the particular circumstances under which it is employed, may result in changes of body economy that are potentially more dangerous without external sign than those with external signs, that prompt the use of the measure.

The data available here constitute an inadequate basis for the predetermination of the entire possible range of satisfactory application of evipal and pentothal in clinical practice; however, by correlating early clinical findings with these experimental data, a more satisfactory, although incomplete, field of use can be estimated than was previously possible.

For abdominal surgery muscular relaxation is desired by the surgeon. This does not mean merely that it is desirable that muscle tonus be decreased, but also that muscular contraction does not follow the sensory nerve stimulation of the surgeon's manipulations. The palpation of the intact abdomen of an animal during anesthesia gives little practical information concerning the degree of relaxation present at a certain depth of

anesthesia. Since contraction of muscle elicited by the surgeon's procedures depends upon the integrity of the reflex arc, a basic approach to the problem must include a determination of the relationship between the depression of reflex excitability and the depression of breathing and circulation per unit of drug.

Experimentally, the reflexly excited contraction of the semitendinosus muscle and the vagopulmonary reflexes excited by lung volume changes were not completely suppressed by evipal or pentothal before respiration failed. At a moderate level of anesthesia there was usually only a relatively small decrease in reflex excitability (muscle, corneal, lid, and vagopulmonary reflexes). However, at a moderate depth of anesthesia the abdomen felt relaxed but a firm, quick, point pressure (tip of finger) was quickly followed by tensing of the muscles under the finger. In short, complete muscular relaxation could not be obtained with evipal or pentothal without unduly endangering life from respiratory failure. This statement is supported by clinical observation: pelvic examinations are unsatisfactory under even deep pentothal anesthesia because of the contraction of the muscles of the abdomen and pelvic diaphragm when manual pressure is applied; relaxation of the anal sphincter and of the rectus muscles does not approach that obtained with spinal anesthesia; and the laryngeal reflexes are depressed sufficiently for tracheal intubation only in deep anesthesia. It is, therefore, probable that a degree of muscular relaxation that is readily and safely attainable with ether or spinal anesthesia is made impossible here by the danger of respiratory arrest. The use of pentothal or evipal for abdominal and much rectal surgery cannot therefore permit the surgeon to work with the facility that is afforded by other agents at a safer level of anesthesia. The range of utility of evipal and pentothal in thoracic surgery as suggested by experimental work is very narrow (2).

The safety of an anesthetic also depends in a large measure upon the constancy of the early signs of overdosage that enable the anesthetist to take appropriate measures before respiratory or circulatory depression becomes severe and consequently more dangerous. Under evipal and pentothal anesthesia the corneal and lid reflexes are

frequently elicitable in dogs and in man in deep anesthesia, and are occasionally present (in dogs) after respiratory arrest has been produced. With evipal, some depression of costal breathing usually takes place before respiratory arrest (Figures 1 to 4); this occurs infrequently with pentothal (Figures 5 to 8). A decrease in minute volume of breathing that can be grossly detected does not occur with constancy between light and deep levels of anesthesia. It is not unusual to have the rate and minute volume of breathing increase as more drug is given, especially if anoxia is present. The eye signs and respiratory signs that ordinarily help guide the anesthetist are not sensitive enough or constant enough under these agents, in the various circumstances that may be encountered, to use them as a very dependable gauge in determining the depth of anesthesia or the imminence of respiratory arrest. Furthermore, if anoxia is present, overdosage is frequently completely masked until an acute and severe respiratory depression develops (1). Overdosage is also masked by sensory nerve stimulation (17), and partial pneumothorax (18). An anesthetist is therefore severely handicapped when employing these agents, largely because evipal and pentothal do not adequately depress certain reflexes before a dangerously deep level of anesthesia has been attained.

It is hoped that this brief consideration of some of the difficulties to be surmounted before more satisfactory methods of preclinical evaluation can be established, may lead to an examination of all commonly used or new anesthetics that will eventually enable the anesthetist to choose accurately an anesthetic that is suitable for the patient and the surgeon, without unduly endangering the life of the former or raising the ire of the latter.

It is suggested that a more valuable preclinical evaluation of anesthetics from the standpoint of respiration could be made if laboratory studies were extended to include, besides their effects on pulmonary ventilation, simultaneous determinations of their effects upon anoxic (chemoreflex control) and hypercapnic (chemoreflex and central control) hyperpnea, upon the hypopnea that is associated with hyperoxia with some anesthetics⁶ (as a means of measuring roughly the func-

⁶ Evipal (1), pentothal (1), cyclopropane (19).

tional degree of anoxic chemoreflex activity at any depth of anesthesia), upon the Hering-Breuer reflexes under normal and high oxygen tensions (to obtain a rough idea of the changing relationship of vagopulmonary reflexes to the central mechanism), upon sphincteric, ocular, and muscle-proprioceptive reflexes (to obtain some evidence as to the effect of the agent upon general reflex excitability), and upon nociceptive excitatory effects on breathing (to determine the masking effect of painful stimuli upon the respiratory signs of depth of anesthesia). A correlation of the data obtained from such experiments seems to afford, as illustrated in this paper, some hope that a successful approach may be found to the problem of the more accurate preclinical evaluation of anesthetic agents.

SUMMARY AND CONCLUSIONS

The evidence gained from these experiments has been interpreted to indicate that:

The breathing of a normal dog lightly anesthetized with evipal is primarily regulated by carbon dioxide, and that the respiratory exchange necessary to effect the removal of carbon dioxide in some animals at rest is apparently greater than that necessary to maintain an oxygen tension of the arterial blood that will keep the chemoreceptor activities at a level which renders their influence generally undetectable in the intact organism.

An increased depth of evipal or pentothal anesthesia can abolish entirely the center's sensitivity to carbon dioxide, but does not greatly affect the intensity of reflex response to a given hypoxic (chemoreceptive) or physical (vagopulmonary proprioceptive) stimulus. Therefore, under moderately deep evipal or pentothal anesthesia the maintenance of breathing rests in large measure if not entirely upon the peripheral chemoreceptive reflex mechanisms. Respiratory adjustments are made mainly upon the basis of the oxygen need of the carotid and aortic bodies and are not directed toward maintenance of a normal acid-base balance, for a large increase in carbon dioxide tension of the arterial blood is unattended by any change in breathing when the center is sufficiently depressed. However, these same bodies, when subjected to oxygen lack, effect in many instances

a greater increase in breathing when the carbon dioxide sensitivity of the center is low than when it is high.

The finding that a high carbon dioxide tension in the arterial blood is incapable of producing increased respiratory activity at a time when a decreased oxygen tension produces a significant stimulation is *not* construed as evidence that the glomus endings are not being stimulated by carbon dioxide; such an action might well be masked by the depressant effect of carbon dioxide upon reflexes.

The depression of the respiration of dogs by evipal and pentothal anesthesia shows great individual variation. The number of experiments is too small to permit any definition of the possible limits of variability.

The breathing of oxygen during evipal and pentothal anesthesia, although it delays the onset of anoxemia, does not remove the danger of diminished oxidations. The hypopnea associated with hyperoxia permits the development of hypercarbia. The reduction of oxidations due to the carbon dioxide retention may be more dangerous than the direct anoxia suffered when air is breathed, for as a consequence of this anoxia respiration is stimulated and the accumulation of carbon dioxide is greatly retarded.

Muscular relaxation with evipal or pentothal of a degree that is readily and safely obtained with ether is prohibited by the danger of respiratory arrest. The use of pentothal or evipal for abdominal or major rectal surgery therefore does not permit the surgeon to work with the facility that is afforded by other agents at a safe level of anesthesia.

The anesthetist is severely handicapped when employing evipal or pentothal because of the variability of response to these agents with the content of the blood gases and other factors discussed. The inability of the anesthetist to determine the status of the respiratory control with the simple means at his disposal seriously handicaps the use of these agents. Generalizations are made as to appropriate factors to study in evaluating new anesthetic agents.

We thank Miss Anne Murphy, B.S., for her excellent technical assistance.

REFERENCES

1. Beecher, H. K., and Moyer, C. A., Mechanisms of respiratory failure under barbiturate anesthesia (evipal, pentothal). *J. Clin. Invest.*, 1941, 20, 549.
2. Moyer, C. A., Major changes in the fundamental relationships of the respiratory drive mechanisms during evipal and pentothal anesthesia, with special consideration of possible applications to transpleural surgery. *J. Thoracic Surg.*, 1941, 11, 131.
3. Henderson, Y., *Adventures in Respiration*. Williams and Wilkins Co., Baltimore, 1938.
4. Marshall, E. K., Jr., and Rosenfeld, M., Depression of respiration by oxygen. *J. Pharmacol. and Exper. Therap.*, 1936, 57, 437.
5. Comroe, J. H., Jr., and Schmidt, C. F., Part played by reflexes from carotid body in chemical regulation of respiration in dog. *Am. J. Physiol.*, 1938, 121, 75.
6. Haldane, J. S., *Respiration*. Yale University Press, New Haven, Conn., 1927.
7. Schmidt, C. F., and Comroe, J. H., Jr., Functions of carotid and aortic bodies. *Physiol. Rev.*, 1940, 20, 115.
8. *MacLeod's Physiology in Modern Medicine*. Edited by Bard, P., C. V. Mosby Co., St. Louis, 1938 and 1941, eds. 8 and 9. See section on the respiration by C. F. Schmidt.
9. Cordier, D., and Heymans, C., *Le Centre Respiratoire*. Hermann et Cie., Paris, 1935.
10. Gesell, R., Respiration and its adjustments. *Ann. Rev. Physiol.*, 1939, 1, 185.
11. Gesell, R., A neurophysiological interpretation of the respiratory act. *Ergebn. d. Physiol.*, 1940, 43, 477.
12. Samaan, A., and Stella, G., Response of chemical receptors of carotid sinus to tension of CO₂ in arterial blood in cat. *J. Physiol.*, 1935, 85, 309.
13. von Euler, U. S., Liljestrand, G., and Zotterman, Y., Excitation mechanism of chemoreceptors of carotid body. *Skandinav. Arch. f. Physiol.*, 1939, 83, 132.
14. Glazer, W., Regulation of respiration; effects of mechanical asphyxia and administration of carbon dioxide, sodium carbonate, sodium bicarbonate and sodium cyanide on reflex response of anterior tibial muscle of dog. *Am. J. Physiol.*, 1929, 88, 562.
15. King, C. E., Garrey, W. E., and Bryan, W. R., Effect of carbon dioxide, hyperventilation, and anoxemia on knee jerk. *Am. J. Physiol.*, 1932, 102, 305.
16. Gesell, R., and Moyer, C., Is breathing fundamentally a reflex phenomenon? *Quart. J. Exper. Physiol.*, 1935, 25, 13.
17. Beecher, H. K., and Moyer, C. A., Central stimulation of respiration during hypoxia. *Am. J. Physiol.*, 1942, 136, 13.
18. Moyer, C. A., and McKittrick, J. B., Unpublished data.
19. Shackell, L. F., and Blumenthal, R. R., Gaseous anesthetics; the effect of cyclopropane on healthy and tubercular rhesus monkey. *Anesth. and Analg.*, 1934, 13, 133.

THE ANTICOAGULANT EFFECTS IN RABBITS AND MAN OF THE INTRAVENOUS INJECTION OF SALTS OF THE RARE EARTHS

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The recent interest in the anticoagulant action of heparin has led us to seek a substance which might be similar in action but less expensive and simpler to administer. The anticoagulant effect of various rare earths given intravenously has been studied in lower animals (1, 2, 3). As far back as 1920, Esnault and Brou (4), and Grenet and Drouin (5) injected rare earths in 40 to 100 mgm. doses intravenously into human subjects, as therapeutic agents in tuberculosis. More recently, Dyckerhoff and Goossens (6) found that neodymium salts injected intravenously prolonged (considerably) the coagulation time of the blood of dogs and man. These authors gave some 30 injections to human subjects, the total dosage of neodymium salt varying from 250 to 500 mgm. The anticoagulant action persisted for as long as 6 hours after a single injection, and no ill effects were noted. No anticoagulant effects resulted from the oral or subcutaneous administration of neodymium.

The need for a cheaper and more easily administered anticoagulating agent than heparin, led us to study the anticoagulant properties of rare earths. The experimental data which we obtained on animals justified an attempt to observe the effects of these substances in man. Our experience, which does not support the claims of Dyckerhoff and Goossens that they are harmless to man, is the subject of this report.

I. Preparation of salts of rare earths for intravenous injection: Neodymium nitrate was dissolved in distilled water or 0.4 per cent saline to a concentration of 0.8 to 2.0 per cent. Neodymium acetate was prepared as follows: To a solution of neodymium nitrate an excess of concentrated NH_4OH was added, precipitating the blue-white finely divided hydroxide. This was dissolved in concentrated acetic acid, and the solution evaporated to the gummy neodymium acetate which dissolved readily in distilled water to make 1 to 2 per cent solutions. The neodymium lactate was similarly prepared by adding concentrated lactic acid to dissolve the hydroxide and dilute NaOH or NaHCO_3 was added to make the solution just

pink to phenol red. The resulting solution was filtered before use and diluted to a 1 to 2 per cent solution. All the solutions were autoclaved just before use.

II. Methods of investigation: The coagulation time of whole blood and plasma was determined by the Lee and White method (7). Serum neodymium was determined as follows: Since neodymium oxalate is of the same low order of solubility as calcium oxalate, the method of Clark and Collip (8) for the determination of calcium could be adapted to our purposes. This method precipitates the oxalate-insoluble salts of serum (usually only calcium, but after injection of neodymium, both calcium and neodymium) and titrates with 0.1 N KMnO_4 to an accuracy of 0.1 mgm. in a total of about 10 mgm. calcium per 100 cc. of serum. In our patients, neodymium concentration is determined as the difference between the "blood calcium" before and during the experiment. Urine neodymium was determined by precipitating all of the insoluble oxalates from an aliquot part of a 24-hour specimen: After separating and washing the precipitate, it was ignited (oxidizing the oxalate), redissolved in nitric acid, and neutralized by excess NH_4OH . The neodymium hydroxide in contrast to the calcium hydroxide is insoluble. Plasma hemoglobin was determined by the benzidine method as previously used in this hospital (9). Red cell fragility was performed by a quantitative method (10). Urine albumin was determined as the urine total protein by a gravimetric, trichloroacetic precipitation test.¹

RESULTS

I. In vitro studies of the effect of neodymium on rabbit plasma

Oxalated normal rabbit plasma, recalcified with an excess of a 0.28 per cent solution of CaCl_2 , was found to have a coagulation time of 5 minutes. Addition of 0.1 cc. of distilled water to 0.1 cc. of such plasma resulted in a coagulation time of $4\frac{3}{4}$ minutes; when, however, 0.1 cc. of solutions of neodymium nitrate in distilled water of 0.003 per cent, or greater concentration, was added to 0.1 cc. of such recalcified plasma, the coagulation time was prolonged (Table I).

¹ We are indebted to Doctors M. D. Altschule and D. R. Gilligan for performing the plasma hemoglobin, red cell fragility, and urine albumin determinations recorded in these experiments.

TABLE I

The effect of 0.1 cc. of $Nd(NO_3)_3$ solution on the coagulation time of 0.1 cc. of recalcified oxalated rabbit's plasma

Concentration of $Nd(NO_3)_3$ mgm. per 0.1 cc.	Plasma coagulation time minutes
0.003	4.25
0.004	6.0
0.006	4.75
0.008	5.5
0.013	7.5
0.017	8.0
0.025	No coagulation resulted but fine particles appeared along sides of test tube after a considerable interval.
0.033	
0.050	

II. *In vitro* studies of the effect of neodymium on human plasma

Oxalated recalcified normal human plasma was found to have a coagulation time of $5\frac{1}{2}$ minutes. When 0.1 cc. of distilled water was added to 0.1 cc. of such plasma the coagulation time was 6 minutes; when, however, 0.1 cc. of solutions of neodymium nitrate in distilled water of 0.003 per cent, or greater concentration, was added to 0.1 cc. of such plasma, the coagulation time was prolonged (Table II).

TABLE II

The effect of 0.1 cc. of $Nd(NO_3)_3$ solution on the coagulation time of 0.1 cc. of recalcified oxalated human plasma

Concentration of $Nd(NO_3)_3$ mgm. per 0.1 cc.	Plasma coagulation time minutes
0.003	6.0
0.004	6.5
0.006	8.25
0.008	9.5
0.013	21
0.017	24
0.025	No coagulation resulted but fine particles appeared along sides of test tube.
0.033	
0.050	

III. *In vivo* studies in the rabbit

Several groups of rabbits were given various amounts of a 5 per cent solution of neodymium nitrate intravenously, all the animals in a given group receiving the same amount; blood samples, secured from the heart by a long needle and syringe previously rinsed in normal saline, were taken from one or more rabbits at varying intervals up to 6 hours after injection.²

² This procedure was necessitated by the fact that in the presence of a prolonged coagulation time, cardiac

The average coagulation time after each dose (Figure 1) was increased, being greatest in the first hour or two after injection, following which it declined. The coagulation time and the duration of delayed coagulability varied with the dose injected. When the clotting time was prolonged beyond 3 to 5 hours, the end point was indefinite and a true clot did not form for days; instead, fine masses of coagulum, less than 1 mm. in diameter, were deposited on the sides of the tube.

Toxicity. Since the solutions seemed to cause restlessness in rabbits, an effort was made to complete the injection as rapidly as possible. In later experiments the rate of injection was decreased to 1 cc. per minute. During rapid injection of smaller doses or slower injection of large doses, whining or restlessness occurred and were followed by death before the end of injection. In smaller dosage some rabbits developed

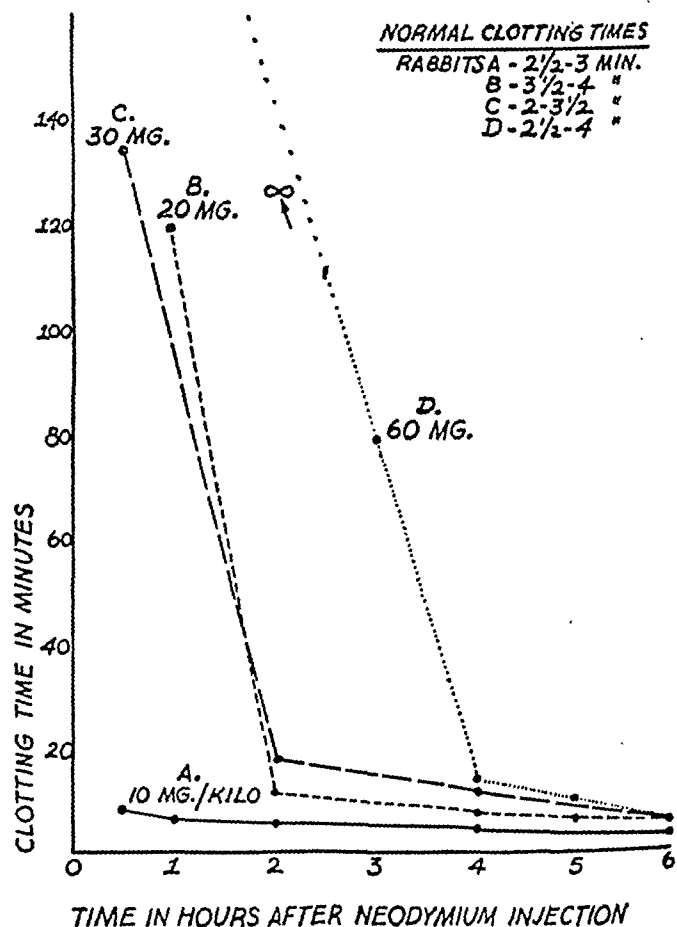


FIG. 1. THE ANTICOAGULANT EFFECTS OF VARYING DOSES OF NEODYMIUM IN RABBITS

puncture caused fatal hemopericardium so often that more than two successive samples from a given rabbit were usually not procurable.

opisthotonos and thrashed their feet about, usually dying within 30 minutes. Others appeared limp and stunned, recovering partially after 10 to 30 minutes, only to die 24 to 48 hours later. One rabbit which received 80 mgm.,³ slowly injected, showed flaccid paralysis of the legs 3 days later. The dosage producing these toxic effects varied considerably, as little as 50 to 80 mgm. causing immediate death if injected rapidly. Some rabbits survived after 80 to 120 mgm. doses, while others lived for as long as 24 to 48 hours after slowly injected doses as high as 200 mgm.

The irritating effect of neodymium nitrate solution resulted in phlebitis within 24 hours after injection, with edema and subsequent necrosis of the rabbit's ear. When the clotting time was markedly prolonged, the site of injection bled persistently and marked anemia occurred.

The toxicity of sublethal doses, repeated over an extended period of time, was studied in 2 rabbits.

One rabbit received 80 mgm. of $\text{Nd}(\text{NO}_3)_3$ daily for 2 days. Two days after the second injection, the animal had a flaccid paralysis of all 4 limbs and was sacrificed.

At autopsy, the only gross findings were a few white millet-seed sized firm nodules in the liver. On microscopic examination, the liver showed small round cell infiltrations about the periphery of the lobules; the lungs showed a few small areas of atelectasis about bronchioles and a few patchy areas of pneumonitis. The brain and remaining organs were essentially normal.

A second rabbit received 840 mgm. of $\text{Nd}(\text{NO}_3)_3$ in doses of 50 to 100 mgm. daily, over a period of 12 days, with no untoward manifestations. It was sacrificed on the 12th day. Autopsy was negative.

IV. *In vivo* studies in man

Having established a minimum lethal dose in rabbits, having confirmed the innocuousness of small anticoagulant doses in animals as previously reported, and having found no reference to severe untoward effects in man, we decided cautiously to administer neodymium to humans. Accordingly, 18 patients were given intravenous doses of neodymium far below the expected levels of toxicity as determined in animals. These patients were mainly in the first postoperative week of various surgical procedures, so chosen because of the oc-

casional occurrence of phlebothrombosis in this period, and because of the recently proposed use of heparin routinely as prophylaxis against this condition.

An anticoagulant effect was produced by single intravenous doses varying from 4.5 to 12.5 mgm. of neodymium salt per kilogram of body weight (Table III).

TABLE III

The anticoagulant effect of single injections of neodymium acetate in man

Subject number	Total	Dose	Clotting time before injection	Interval after injection	Clotting time after injection
	mgm.	mgm. per kgm.	minutes	minutes	minutes
1	200	3	10	15	12.5
2	400	6	11	20	20
3	400	7	12	20	11
4	600	7.5	11	20	16
5	640	8	11.5	20	15
6	1000	12.5	12	24	42
7	800	9.75	15	20	20
8	250	4.5	16	67	24
9	622	10	12	67	20

Doses of more than 10 mgm. produced an anticoagulant effect which reached a peak in $\frac{1}{2}$ to $1\frac{1}{2}$ hours, followed by a decline. Small additional doses of neodymium given during the phase of decreasing clotting time caused a substantial increase in clotting time (Figure 2). One dose of 18 mgm. resulted in an indefinitely prolonged clotting time, $3\frac{1}{2}$ hours after injection (Figure 3). About 5 hours after injection when the clotting time was 50 minutes, gross hematuria was noted.

Toxicity. Because neodymium nitrate solution caused thrombophlebitis at the site of injection in man, the acetate and lactate salts of neodymium were substituted. The latter salts produced a painless thrombosis of the injected vein after 24 to 48 hours in about a fourth of the cases, when total doses of 300 mgm. and over were given. After 10 of 17 injections, fever ranging as high as 101° occurred 3 to 18 hours following administration. Headache, sweating, muscle and abdominal pains, nausea, and vomiting also occurred. These symptoms were unrelated to the degree of anticoagulant effect, thrombophlebitis, or hemoglobinemia (see below) and were considered to be toxic manifestations of the drug. Moreover, no one patient had all the aforementioned side effects and several patients had none.

³ All doses referred to in this paper are in terms of the weight of the rare earth salt per kilogram.

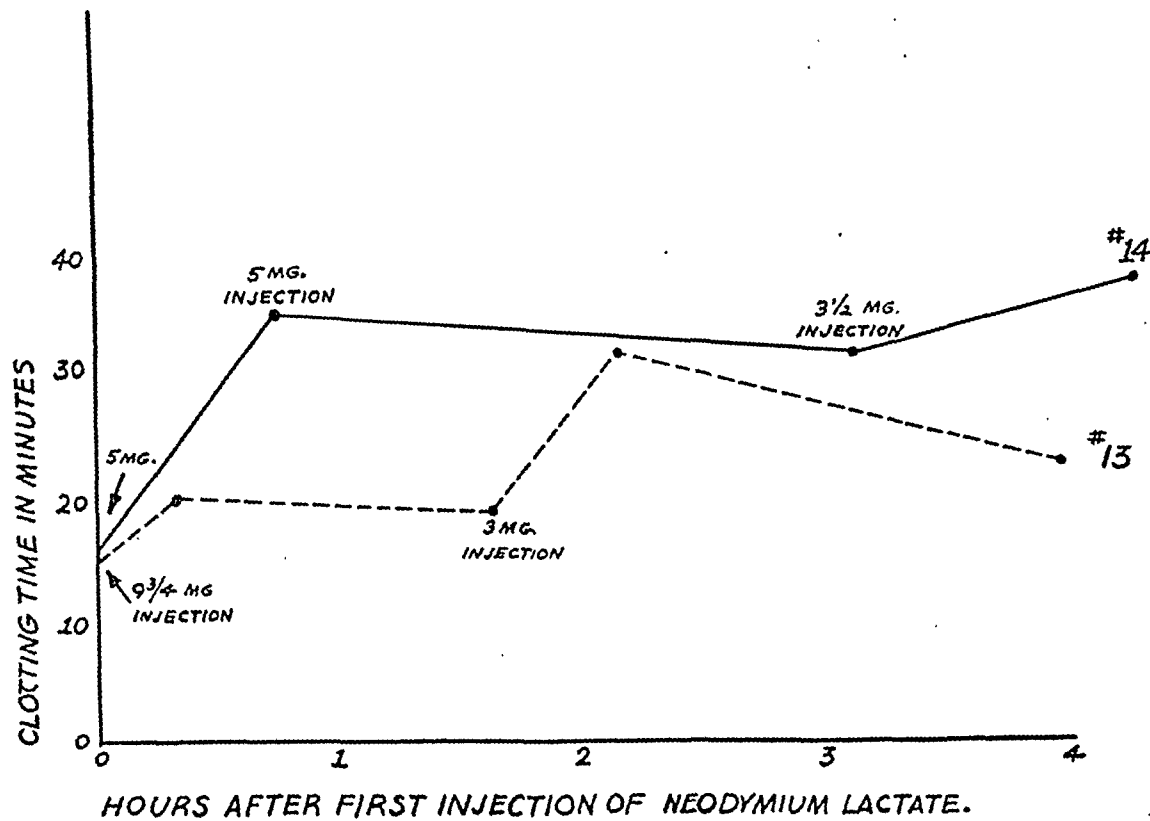


FIG. 2. THE ANTICOAGULANT EFFECT OF REPEATED DOSES OF NEODYMIUM IN MAN

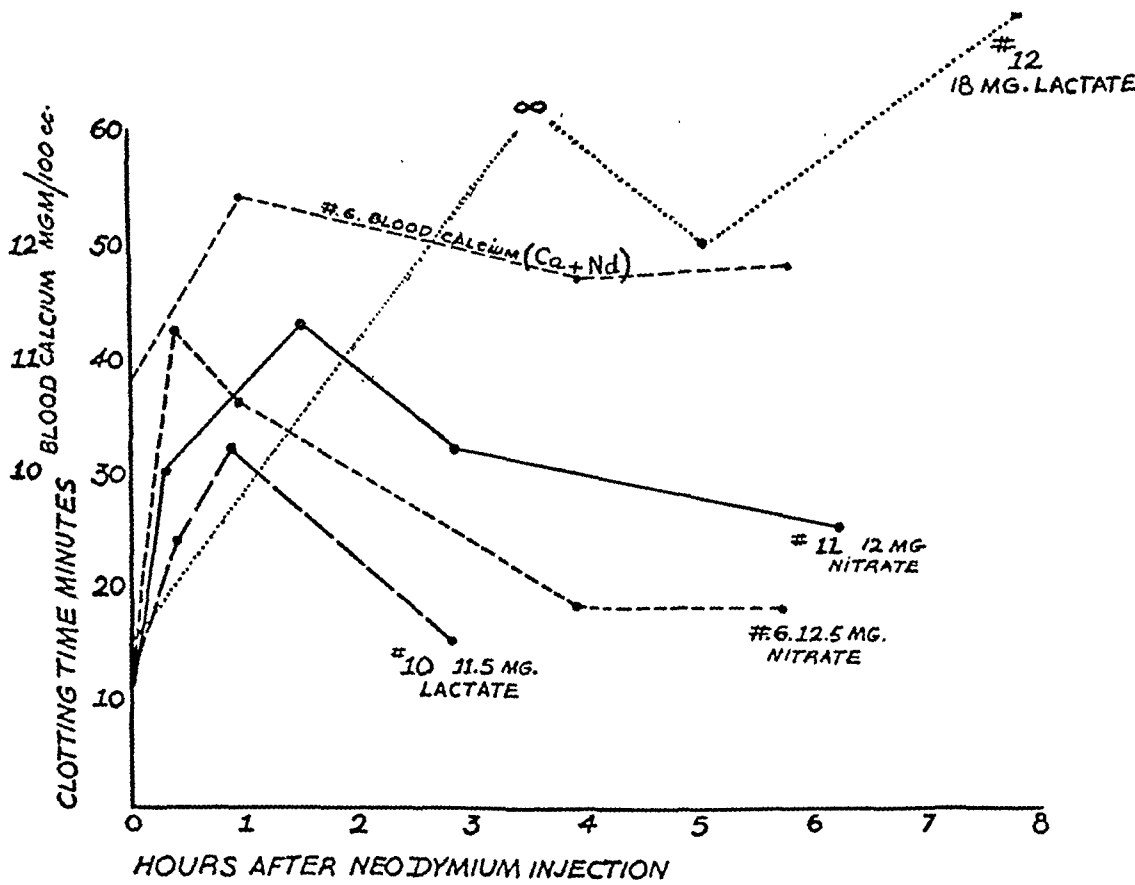


FIG. 3. THE ANTICOAGULANT EFFECT OF VARYING DOSES OF NEODYMIUM IN MAN

Other rare earths, lanthanum acetate and cerium acetate, were given in doses varying from 1.6 to 5 mgm. Because similar but more severe toxic symptoms resulted even before anticoagulant doses were reached, further use of these drugs was not attempted.

An attempt was made to avoid toxic reactions by altering the mode of administration, as suggested by Greenbaum and Aye (11) who noted a reduction in the toxicity of thorium salts in rabbits after dissolving the salts in 50 per cent sucrose solution. Accordingly, one patient was given 9.5 mgm. of neodymium in a 50 per cent sucrose solution but toxic symptoms were not prevented.

Two patients were given daily doses increasing from 1 to 6 mgm., over a period of a week in an attempt to obviate toxic effects, as suggested by Dyckerhoff and Goossens (6). There were no untoward symptoms, but hemoglobinemia resulted.

Hemoglobinuria occurred as a toxic manifestation after injection of neodymium in man. Of 10 patients who received single doses from 1.2 to 1.8 mgm., 5 showed plasma hemoglobin values above the normal of 9 mgm. per cent, for 1 to 3 hours after injection. In 3 patients, plasma hemoglobin levels suggesting the breakdown of 10 to 20 cc. of blood were found. In another instance, the plasma hemoglobin was 17 mgm. per 100 cc., 24 hours after injection of neodymium; it was not possible to estimate the degree of hemolysis in this instance. In one experiment, the patient had a moderate headache the morning after receiving an initial dose of 11.5 mgm. An additional dose of 14 mgm. was then given; 1 hour later the clotting time was over 8 hours and the plasma was normal in color. Two hours after injection the patient voided a dark red urine and complained of severe abdominal pain which recurred several times during the next 24 hours. The body temperature did not rise above 99.8°. The red urine persisted for 2 days and on the third day, when the urine was of normal color though still positive to benzidine, the blood showed a plasma hemoglobin of 143 mgm. per 100 cc., and a bilirubin of 1.1 mgm. per 100 cc. The fragility of the red cells at this time was normal and the total blood hemoglobin and erythrocyte count were the same as before injection. The plasma hemoglobin

reached normal 5 days after the onset of hemoglobinemia. Probably several hundred cc. of blood had been hemolyzed.

Another patient who showed hemoglobinuria had received progressively increasing doses of neodymium lactate over a period of a week (Figure 4). The last dose resulted in a definite anticoagulant effect; no toxic symptoms occurred. A light mahogany colored urine was voided on the morning of the seventh day; this specimen showed strongly positive benzidine and guaiac tests. There were granular casts and 1 to 4 red blood cells and white blood cells in centrifuged specimens of the darkest urines. The casts contained large granules which turned black on the application of $(\text{NH}_4)_2\text{S}$ solution indicating the presence of hemoglobin pigment. No anemia resulted from this attack of intravascular hemolysis. There was no clinical jaundice and the plasma bilirubin increased only slightly.⁴

Hemolysis was not produced in vitro when 1 mgm. of neodymium per cc. was added to human blood which was allowed to stand at room temperature and at 37.5° C. for as long as 2 hours; nor was it produced in one dog given 50 mgm. of neodymium nitrate per kilo.

DISCUSSION

The anticoagulant effect of neodymium nitrate in rabbits was found to be similar to that reported in dogs (3). The minimal effective dose was about 10 mgm. per kgm. of body weight and the lethal dose was 80 mgm. or more.

The anticoagulant effects of neodymium salts in man show a close resemblance to those in rabbits. When either rabbit or human whole blood was tested, 1.25 to 2.0 mgm. of neodymium salt were required to prevent 1 cc. of blood from clotting indefinitely. Raising the dose increases both the clotting time and the duration of the effect. The minimal effective dose in man varies from 5 to 8 mgm. per kgm. The increase in coagulation time reaches a peak within an hour or so, and then starts to decrease; the clotting time can be prevented from returning to normal by administration of successive smaller doses. When the dose is increased above 14 mgm., the blood is rendered

⁴ This may be used roughly as an index of the normal functional state of this patient's liver (after receiving 20 mgm. per kgm. of neodymium), in view of its ability to handle blood pigments released intravascularly.

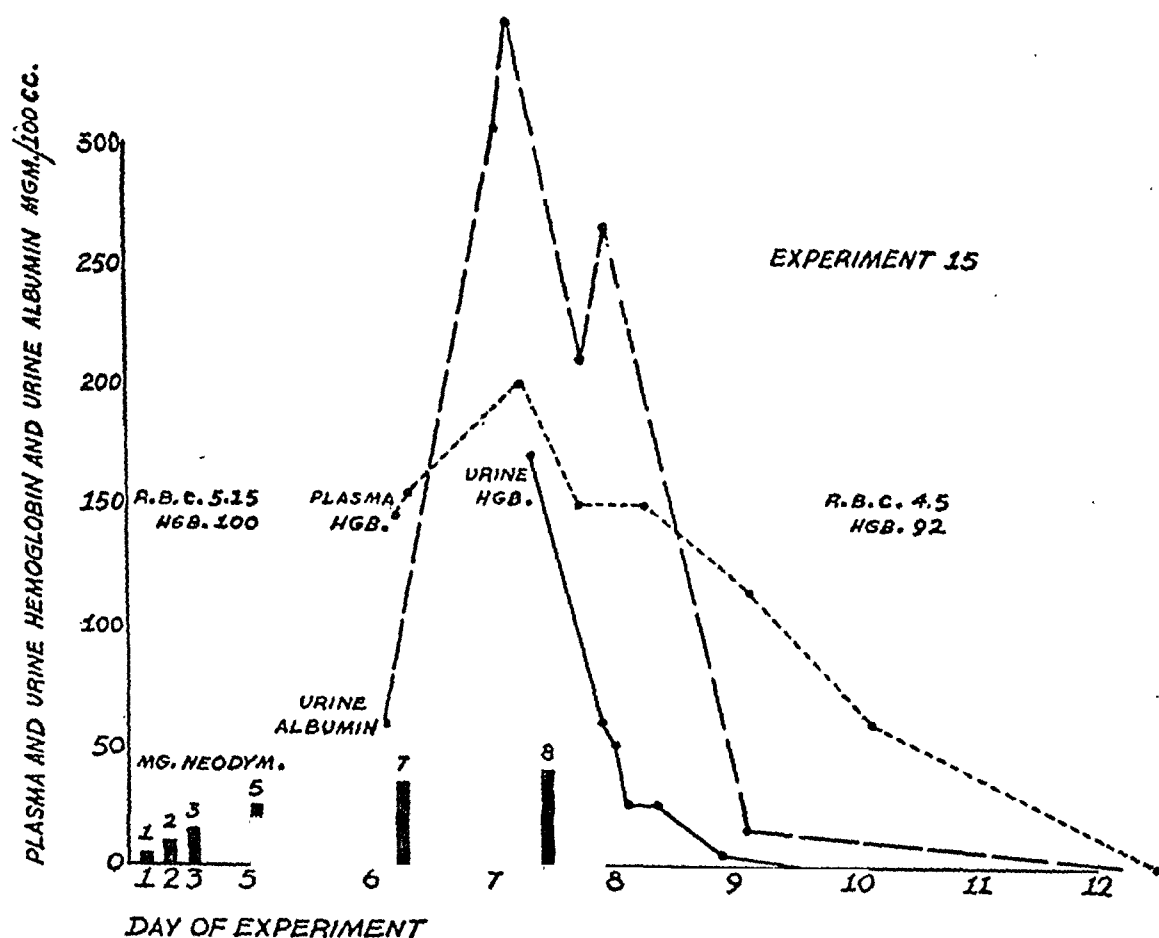


FIG. 4. THE COURSE OF HEMOGLOBINEMIA AND HEMOGLOBINURIA IN MAN FOLLOWING NEODYMIUM

uncoagulable. As an example of the range of values involved, a single dose of 12 mgm. increased the clotting time to 2 to 4 times normal for a period of over 6 hours.

All of the rare earths are anticoagulant substances (3). The mode of action is not completely understood but is independent of the anions used (12). They do not act by interfering with the coagulant effect of calcium (13). Glazko and Greenberg, who studied the activity of thorium salts in vitro, concluded that, "the inhibiting action of polyvalent cations was found to be due to their effect on fibrinogen" (14), in contrast to polyvalent anions and heparin which act on prothrombin.

The problem is made more complicated by the fact that the dose required to render blood uncoagulable indefinitely in dogs (3) is greater in vitro than in vivo. This ratio was found to be about 10 to 1 in man.⁵ Moreover, the action of

the drug in vivo requires 30 to 120 minutes to reach a maximum, whereas in vitro the effect is immediate.

It has been shown that the concentration of either prothrombin (15) or fibrinogen (16) in plasma must be reduced by fully 80 per cent before clotting time is measurably prolonged. Moreover, after the prothrombin concentration has been reduced to this critical level, any further reduction causes a rapidly increasing clotting time (15). This resembles the sequence of events which appears when neodymium inhibits the activity of fibrinogen in blood. Study of the action of neodymium both in animals and man shows that after the minimal dose necessary to prolong the clotting time has been given, further administration of relatively small doses causes rapid prolongation of the clotting time.

a 6000 cc. blood volume required 1000 mgm. of neodymium lactate to render blood withdrawn one hour after injection uncoagulable indefinitely. This is equivalent to 17 mgm. per 100 cc.

⁵ In vitro, about 125 to 200 mgm. is required per 100 cc. of blood. By contrast, a patient with approximately

A COMPARISON OF PITUITRIN WITH THE ANTIDIURETIC SUBSTANCE FOUND IN HUMAN URINE AND PLACENTA

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An antidiuretic substance was found by Gilman and Goodman (1) in the urine of normal rats during dehydration, whereas hypophysectomized rats failed to excrete this substance even during dehydration of lethal grade. Their hypothesis that the antidiuretic substance has its origin in the pituitary gland has been both confirmed (2, 3) and questioned (4, 5). Arnold (4) and Walker (5) found that hypophysectomy failed to reduce uniformly the antidiuretic activity of the urine of dehydrated animals. From this and other evidence it has been suggested that the antidiuretic substance of urine has certain properties differing from those of pituitrin and does not originate in the pituitary gland.

The urine of patients with toxemia of pregnancy contains an antidiuretic substance according to Teel and Reid (6) and Krieger and Kilvington (7). In edema of various types Robinson and Farr (8) found that the antidiuretic activity of the urine was high while fluid was being retained, and low after diuresis had occurred. While Anselmino, Hoffman and Kennedy (9) were able to isolate an antidiuretic and pressor pituitrin-like substance from the blood of patients with toxemia of pregnancy, others have consistently been unable to do so (10 to 16).

The antidiuretic substance found in urine deserves further study, not only because its origin appears to be uncertain, but also because of the important role it might conceivably play in those forms of water retention and edema which so far have not been adequately explained by the purely physical forces included in the Starling hypothesis. The studies reported in this paper (a) confirm the presence of an antidiuretic substance in the urine of patients with toxemia of pregnancy, (b) describe differences between this substance and pituitrin with respect to dialysis, ultracentrifugation, and effect on chloride excretion, and (c) demon-

strate that placentas from patients with toxemia of pregnancy contain an antidiuretic substance which resembles in certain respects that found in their urine.

METHODS AND MATERIAL

The antidiuretic substance studied in these experiments was, with the few exceptions noted below, obtained from the urine of patients admitted to the Obstetrical Service² of the Hospital of the University of Virginia, because of hyperemesis gravidarum, hydatid mole, preeclampsia and eclampsia. Urine was collected over accurately timed periods averaging 12 hours. Before and during the collection of urine, patients received adequate amounts of fluid; therapeutic dehydration was delayed until the collection was complete.

Contamination of the urine with feces was avoided, by catheterization if necessary. Immediately after micturition the individual samples were placed in clean glass bottles in a refrigerator, no acid being added. At the end of the 12-hour collection period, the individual samples were pooled. Specific gravity, reaction to litmus, and a rough quantitative estimation of protein were recorded. In some cases the urinary protein was removed by adjusting to pH 4.0 with glacial acetic acid and heating to 80° C. for 20 minutes, followed by centrifugation and decantation of the supernatant urine. Whether protein was removed or not, the entire specimen was dialyzed against running tap water in an oscillating apparatus containing long cellophane tubes 24/32 inch in diameter and 0.00072 inch thick. Three hours of this dialysis reduced the chloride concentration to undetectable amounts. The dialyzed urine was then concentrated to a volume such that 1 cc. represented the urine excreted in 15 to 25 minutes, by boiling the entire specimen *in vacuo* at 50° C. To reduce the volume of the specimen to the desired amount required about 3 hours. The concentration of chloride at this point ranged between 1 and 25 m.eq. per liter. The urine concentrate was then stored at 5° C. for assay on the following day.

The urine of one normal dehydrated subject was treated similarly after collection during the last 12 hours of a 48-hour period with an intake of 800 cc. per 24 hours. Urine from hydrated and dehydrated rats was collected

¹ Commonwealth Fund Fellow, 1940-41.

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8. Hawk, P. B., and Bergeim, O., *Practical Physiological Chemistry*. P. Blakiston's Son and Co., Philadelphia, 1927, p. 408.
9. Gilligan, D. R., Altschule, M. D., and Katersky, E. M., Studies of hemoglobinemia and hemoglobinuria produced in man by intravenous injection of hemoglobin solutions. *J. Clin. Invest.*, 1941, 20, 177.
10. Hunter, F. T., A photoelectric method for the quantitative determination of erythrocyte fragility. *J. Clin. Invest.*, 1940, 19, 691.
11. Greenbaum, F. R., and Aye, C. E., The pharmacological behavior of sodium thorium tartrate and its roentgen diagnostic value. *Am. J. Roentgenol.*, 1941, 45, 265.
12. Fischler, F., Über experimentelle Beeinflussung der Leberfunktionen und der Anatomischen Leberstruktur durch Einwirkung seltener Erden. *Arch. f. Exper. Path. u. Pharmacol.*, 1938, 189, 4.
13. Dyckerhoff, H., von Behm, W., Goossens, N., and Miehler, H., Über die Gerinnung des Blutes. *Biochem. Ztschr.*, 1936, 288, 271.
14. Glazko, A. J., and Greenberg, D. M., The mechanism of the inhibiting effect of electrolytes and heparin on blood coagulation. *Am. J. Physiol.*, 1940, 128, 399.
15. Quick, A. J., The coagulation defect in sweet clover disease and in the hemorrhagic chick disease of dietary origin. *Am. J. Physiol.*, 1937, 118, 260.
16. Nygaard, K. K., *Hemorrhagic Diseases: Photo-Electric Study of Blood Coagulability*. C. V. Mosby Co., St. Louis, 1941, p. 139.
17. Oelkers, H. A., and Vincke, E., Zur Pharmakologie der seltenen Erden; Wirkung auf das Blutbild. *Arch. f. Exper. Path. u. Pharmacol.*, 1938, 188, 53.
18. (a) Witts, L. J., Heparin in subacute bacterial endocarditis. *Brit. M. J.*, 1940, 1, 484.
(b) Ershler, I. L., and Blaisdell, I. H., Massive hematuria following use of heparin in cavernous sinus thrombosis. *J. A. M. A.*, 1941, 117, 927.
(c) de Takats, G., Heparin. *J. A. M. A.*, 1941, 117, 1378.
(d) Ershler, I. L., and Blaisdell, I. H., Heparin for prolonged coagulation time. *J. A. M. A.*, 1941, 117, 2095.
19. Schofield, F. W., Damaged sweet clover: The cause of a new disease in cattle simulating hemorrhagic septicemia and blackleg. *J. Am. Vet. M. A.*, 1924, 64, 553.
20. Holst, W. F., and Halbrook, E. R., A "scurvy-like" disease in chicks. *Science*, 1933, 77, 354.

A COMPARISON OF PITUITRIN WITH THE ANTIDIURETIC SUBSTANCE FOUND IN HUMAN URINE AND PLACENTA

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An antidiuretic substance was found by Gilman and Goodman (1) in the urine of normal rats during dehydration, whereas hypophysectomized rats failed to excrete this substance even during dehydration of lethal grade. Their hypothesis that the antidiuretic substance has its origin in the pituitary gland has been both confirmed (2, 3) and questioned (4, 5). Arnold (4) and Walker (5) found that hypophysectomy failed to reduce uniformly the antidiuretic activity of the urine of dehydrated animals. From this and other evidence it has been suggested that the antidiuretic substance of urine has certain properties differing from those of pituitrin and does not originate in the pituitary gland.

The urine of patients with toxemia of pregnancy contains an antidiuretic substance according to Teel and Reid (6) and Krieger and Kilvington (7). In edema of various types Robinson and Farr (8) found that the antidiuretic activity of the urine was high while fluid was being retained, and low after diuresis had occurred. While Anselmino, Hoffman and Kennedy (9) were able to isolate an antidiuretic and pressor pituitrin-like substance from the blood of patients with toxemia of pregnancy, others have consistently been unable to do so (10 to 16).

The antidiuretic substance found in urine deserves further study, not only because its origin appears to be uncertain, but also because of the important role it might conceivably play in those forms of water retention and edema which so far have not been adequately explained by the purely physical forces included in the Starling hypothesis. The studies reported in this paper (a) confirm the presence of an antidiuretic substance in the urine of patients with toxemia of pregnancy, (b) describe differences between this substance and pituitrin with respect to dialysis, ultracentrifugation, and effect on chloride excretion, and (c) demon-

strate that placentas from patients with toxemia of pregnancy contain an antidiuretic substance which resembles in certain respects that found in their urine.

METHODS AND MATERIAL

The antidiuretic substance studied in these experiments was, with the few exceptions noted below, obtained from the urine of patients admitted to the Obstetrical Service² of the Hospital of the University of Virginia, because of hyperemesis gravidarum, hydatid mole, preeclampsia and eclampsia. Urine was collected over accurately timed periods averaging 12 hours. Before and during the collection of urine, patients received adequate amounts of fluid; therapeutic dehydration was delayed until the collection was complete.

Contamination of the urine with feces was avoided, by catheterization if necessary. Immediately after micturition the individual samples were placed in clean glass bottles in a refrigerator, no acid being added. At the end of the 12-hour collection period, the individual samples were pooled. Specific gravity, reaction to litmus, and a rough quantitative estimation of protein were recorded. In some cases the urinary protein was removed by adjusting to pH 4.0 with glacial acetic acid and heating to 80° C. for 20 minutes, followed by centrifugation and decantation of the supernatant urine. Whether protein was removed or not, the entire specimen was dialyzed against running tap water in an oscillating apparatus containing long cellophane tubes 24/32 inch in diameter and 0.00072 inch thick. Three hours of this dialysis reduced the chloride concentration to undetectable amounts. The dialyzed urine was then concentrated to a volume such that 1 cc. represented the urine excreted in 15 to 25 minutes, by boiling the entire specimen *in vacuo* at 50° C. To reduce the volume of the specimen to the desired amount required about 3 hours. The concentration of chloride at this point ranged between 1 and 25 m.eq. per liter. The urine concentrate was then stored at 5° C. for assay on the following day.

The urine of one normal dehydrated subject was treated similarly after collection during the last 12 hours of a 48-hour period with an intake of 800 cc. per 24 hours. Urine from hydrated and dehydrated rats was collected

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by the method described by Gilman and Goodman (1). The greater volume of urine excreted by the hydrated group was concentrated to the volume excreted over the same period by the dehydrated group, so that the final samples when assayed represented per cc. the same number of minutes of excretion.

For assay of antidiuretic activity, the rat method of Burn (17) was used in modified form. A stock of male albino rats of a Wistar strain were kept in wire-mesh cages, in groups of 3, in a room maintained at an even temperature. They were fed measured amounts of dog chow checkers at the same time each day. Fresh water was available constantly in a standard drinking cup. This regular feeding and standard drinking equipment were found essential for consistent results. Various described modifications of Burn's rat method and various methods of reading the curves (1 to 3, 6 to 8, 18) were compared, and the results (19) caused the adoption of the following technique, because of its greater dependability and accuracy under the conditions imposed.

Prior to each assay 18 rats were fasted for 12 hours but were allowed to have water *ad lib*. The next morning they were placed, by groups of 3, in a bank of 6 metabolism cages. The cages were set over 9-inch chemically clean glass funnels which drained into 50 cc. graduates. Small conical screens in the apices of the funnels prevented feces from falling into the graduates. The rats were weighed and then distributed so that the total weight of the 3 rats in each group did not differ by more than 10 per cent if possible. Two of the 6 groups were used as controls, leaving 4 groups for duplicate assays of 2 unknown solutions.

To begin the assay procedure, each rat was given by gavage a "priming dose" of 0.2 per cent NaCl equivalent to 2.5 per cent of body weight. This 0.2 per cent NaCl solution yielded more consistent results than did gavage with tap water, particularly with respect to excretion of chloride in the urine (19, 21). Gavage could be accomplished by one operator easily in the following manner. The rat's tail was clamped to a block on the table with rubber straps and the body was held perpendicularly by placing tension on the neck with the left hand. A number 18 lumbar puncture needle, from which the point had been ground and to which a drop of solder in the form of a small bougie had been added, could then be passed easily with the right hand along the roof of the mouth and the esophagus. The needle was connected to a 100 cc. burette, containing 0.2 per cent NaCl solution, maintained under pressure of 10 mm. Hg by compressed air. The desired amount of solution could be run in slowly from the burette without injury, providing the rat was firmly held to prevent flexion of the body. Two hours after this "priming dose" was given, all the urine excreted up to that time was discarded. The volume of urine excreted in this preliminary period varied considerably from group to group and seemed to have no significance except to indicate inequality in voluntary water intake.

Each rat was then given a "hydrating" dose of 0.2 per cent NaCl solution, equivalent to 5 per cent of the orig-

inal weight. Simultaneously, the solution to be assayed was injected intraperitoneally in a volume totaling 1 cc. per 100 grams of rat. The control groups were given similar gavage and received intraperitoneally either 0.9 per cent NaCl solution or distilled water, to match the composition of the solutions being tested.

Starting then at zero time for each cage, the cumulative volume of urine excreted by each group of 3 rats was recorded at 15-minute intervals for 3 hours. The recorded volumes were computed in terms of the amount excreted per 100 grams of rat and plotted against time for each cage, as shown in Figure 1. To express the results by a single number, the areas of these curves were measured in square inches, using a planimeter on the original chart in which one inch represented 30 minutes and 1.0 cc. urine. For example, in Figure 1, the control curve enclosed an area (ABCA) of 11.2 sq. in. The curve corresponding to a dose of 1 milliunit of pituitrin per 100 grams of rat had an area (DECD) of 8.7 sq. in.; the difference between the latter and the control was minus 2.5 sq. in., indicating slight antidiuretic activity. Pituitrin in a dose of 5 milliunits reduced the area (FGCF) to 2.4 sq. in.; the difference from the control area was correspondingly greater, minus 8.8 sq. in., and represented moderate antidiuretic activity.

By this method of expressing results, samples having no effect on water excretion would be represented by curves having areas approximately the same as the controls, and their activity would therefore be designated by a small negative or positive number. Samples having antidiuretic activity would be represented by curves having a smaller area than the controls; the nature and degree of their activity would be designated by a negative number. Conversely, samples having diuretic activity would be represented by curves having a larger area than the controls and their diuretic action would therefore be designated by a positive number.

In 130 experiments, the variation between 2 control cages, observed on the same day, averaged 1.56 sq. in. with a maximal variation of 5 sq. in., the latter occurring in only 2 experiments. In this paper, urine concentrates producing numerical values between zero and minus 2 sq. in. are termed "inactive"; between minus 2 and minus 4 sq. in., "slightly antidiuretic"; between minus 4 and minus 9 sq. in., "moderately antidiuretic"; and minus 9 or more sq. in., "markedly antidiuretic."

The urine excreted by each group, during the 3-hour collection period, was analyzed for chloride by the open Carius method of the Volhard titration as described by Van Slyke and Sendroy (20). Chloride excretion was recorded in terms of total microequivalents³ per 100 grams of rat during the entire 3-hour period.

OBSERVATIONS

1. Dependability and limitations of the method

In preliminary observations, using known amounts of pituitrin, it was found that the method

³ Microequivalents = equivalents $\times 10^{-6}$.

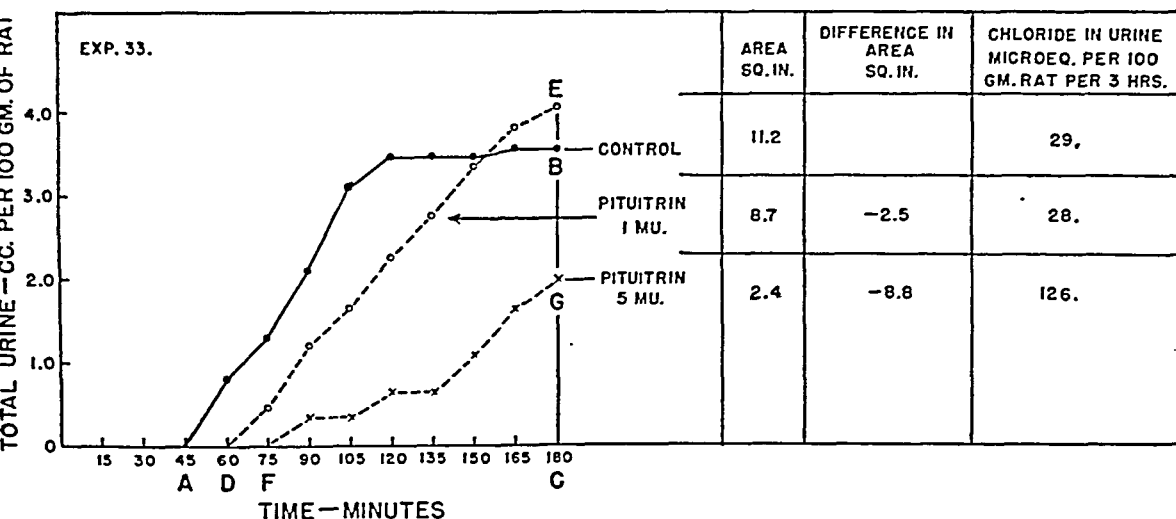


FIG. 1. SHOWING TYPICAL DIURESIS CURVES OF GROUPS OF RATS RECEIVING A CONTROL INJECTION (B), AND 1 (E), OR 5 (G) MILLIUNITS OF PITUITRIN PER 100 GRAMS (For full description see text)

described was quite accurate with doses of 0.5 to 10 milliunits of pituitrin per 100 grams of rat. Doses larger than this produced curves with areas slightly greater than those for 10 milliunits and hence paradoxically smaller numerical values for antidiuresis. Examination of these curves revealed that doses exceeding 10 milliunits per 100 grams of rat produced a brief diuresis and then, secondarily, a profound antidiuresis, whereas the smaller doses produced antidiuresis only. This effect has been described before with larger doses and may be related to the temporary rise in blood pressure produced by pituitrin. Since the expression of results in terms of area includes the total effect, it is essential, with pituitrin at least, to dilute the sample of unknown potency to the proper range to obtain accurate quantitative results.

Although the larger doses of pituitrin had a paradoxically smaller total antidiuretic activity over the 3-hour period chosen, their effect on excretion of Cl in the urine, or their "chloruretic" activity, increased steadily as the dose of pituitrin increased over a range of 0.1 to 100 milliunits per 100 grams of rat. In fact, comparison showed, in agreement with the prediction made by Silvette (21), that pituitrin solutions may be assayed more accurately by measuring their effect on Cl excretion, than by measuring their antidiuretic activity

⁴ Chloruretic: G. chlōros, yellowish green; plus G. ourēsis, urination; urination of chlorides.

alone. These findings apply only indirectly to the problem being reported here and will be described more fully in another paper (19).

Because repeated injections of the colloidal constituents of urine into the same rats at intervals of days or weeks might produce artefacts due to anaphylaxis, it was at first thought necessary to use fresh rats for each assay. However, when the same urine concentrates, with and without protein, were injected into the same rats after various intervals ranging from 7 to 27 days, no difference in the curves of water excretion could be detected, nor was there any gross evidence of anaphylaxis. As a further test 1 cc. of human blood serum was injected into each of 6 rats and repeated 20 days later. The curves of water excretion were the same. Therefore as long as the rats ate well, maintained their weight, and were not obviously abnormal, they were used for not more than 3 assays at intervals of 10 days or more.

2. The antidiuretic activity of human and rat urine under various conditions

The results reported for rats by Gilman and Goodman (1), and for human beings by Teel and Reid (6) and Krieger and Kilvington (7), were confirmed in part (Figure 2 and Table I). No attempt was made to study a large series of cases, these results being listed merely to amplify the reports already published and to describe the source of the material studied.

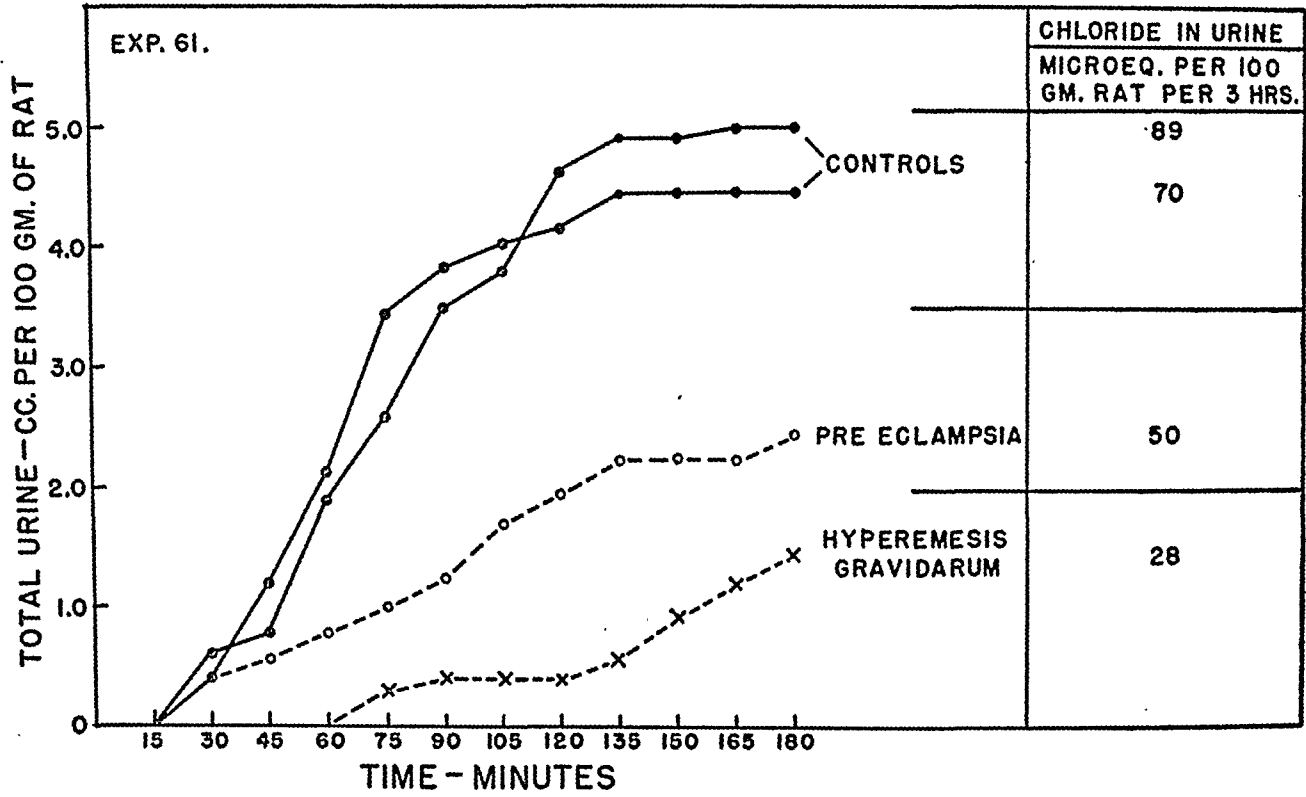


FIG. 2. SHOWING THE ANTIDIURETIC ACTIVITY OF URINE FROM A PATIENT WITH PREECLAMPSIA AND FROM A PATIENT WITH HYPEREMESIS GRAVIDARUM

TABLE I

Showing sources of urine studied, number of cases, and anti-diuretic activity of each specimen

Source of urine		Number of cases	Antidiuretic activity, each specimen			
			None	Slight	Moderate	Marked
Rats	Water <i>ad lib.</i>	2 groups 12 each	2			
	Dehydrated 48 hours	2 groups 12 each			1	1
Normal subjects Male 1, Female 2		3	3			
Normal subject, dehydrated Female 1		1			1	
Chronic glomerulonephritis with edema		2	2			
Preeclampsia		6	1		3	3
Eclampsia		2			1	1
Hyperemesis gravidarum		3			4	2
Hydatid mole		1			1	1

The urine from dehydrated rats was moderately or markedly antidiuretic (Table I), while the urine of rats taking water *ad lib.* was inactive, even though the assay animals received quantities

of dialyzed urine equivalent to the same number of minutes of urinary excretion by the dehydrated rats. The antidiuretic substance in these specimens of rat urine was found to have the same properties as the antidiuretic factor in human urine and will therefore not be discussed separately from this point on.

Three normal human beings, taking water *ad lib.*, did not excrete detectable amounts of antidiuretic substance (Table I), whereas 1 female subject, dehydrated to a moderate degree, excreted a moderate amount of antidiuretic substance. Two patients with chronic glomerulonephritis and edema excreted urine without antidiuretic activity; the edema was explained adequately in these patients by advanced hypoproteinemia. In the remaining 12 cases, all suffering from complications of pregnancy, moderate or marked antidiuretic activity was observed in 16 urine specimens, and no activity in 1 specimen. The cases diagnosed "preeclampsia" presented clinical edema, albuminuria, and some degree of hypertension, while those diagnosed "eclampsia" had, in addition to these abnormalities, at least one generalized convulsion. Figure 2 illustrates the typical results of an assay

of urine from a patient with preeclampsia, and shows a curve indicating moderate antidiuretic activity.

In Figure 2 is included also the result of an assay on the urine of a patient with hyperemesis gravidarum. The presence of antidiuretic substance in the urine of patients with hyperemesis gravidarum and hydatid mole has not been previously reported. The results in Table I indicate that antidiuretic substance appears in the urine of these patients quite uniformly in amounts as great as those found in preeclampsia and eclampsia. In cases of hyperemesis gravidarum, the specimens of urine were obtained during the first 12 hours in the hospital, while fluids were being administered. One patient in this group received, during 72 hours, 6000 cc. of 5 per cent glucose in 0.9 per cent NaCl intravenously. While the urine collected on the first day was markedly antidiuretic, that collected on the third day was only moderately antidiuretic. The presence of antidiuretic substance in the urine of these patients may be merely the result of the dehydration accompanying the condition. The patient with hydatid mole had all the symptoms and signs of severe preeclampsia and the correct diagnosis was made only after the mole was delivered.

3. The effect of dialysis on the antidiuretic activity of pituitrin solutions and of human urine

Gilman and Goodman (1) found that the antidiuretic fraction of pituitrin solution was retained by the dialyzing sacs they used, whereas Walker (5) reported at least a 50 per cent loss of antidiuretic activity when pituitrin, either in saline or in rat urine, was dialyzed. Our findings agree with those of Walker, in that pituitrin added to human or rat urine dialyzed as rapidly as when added to distilled water. There was no evidence that contact with human urine diminished the dialyzability of pituitrin or of its antidiuretic and chloruretic fractions.

Table II illustrates a typical experiment. A pituitrin⁵ solution containing 10 milliunits per cc. in distilled water was injected in the usual dose of 1.0 cc. per 100 grams of rat. This originally produced antidiuresis amounting to minus 7.0 sq. in.

⁵ Obstetrical pituitrin, Parke Davis, lot No. 3260690, 1 cc. containing 10 international units.

but after dialysis for 3 hours, lost its antidiuretic activity completely, producing a curve with an area of plus 2.1 sq. in. In contrast, the antidiuretic substance of urine did not dialyze. To make conditions as similar as possible, 2 aliquots of 1 urine sample were dialyzed, 1 for 3 hours and 1 for 6 hours, then concentrated so that 1 cc. of each specimen corresponded to 24 minutes of urine secretion. They were assayed on the same day for comparison with each other and the same controls. As shown in Table II, after the routine 3-hour dialysis required to free the urine of salts, the first sample produced antidiuresis amounting to minus 5.5 sq. in. Further dialysis of this urine for an additional 3 hours did not change its antidiuretic activity significantly, the figure at 6 hours being minus 6.2 sq. in.

The effect of pituitrin on chloride excretion was also decreased by dialysis from 239 microequivalents per 100 grams of rat to 71 microequivalents per 100 grams, the latter being within the normal range of the controls. As usual (compare Figures 1 and 2) dialyzed urine, even when conspicuously antidiuretic, had no effect on chloride excretion at any time.

Similar results were obtained in 6 such comparisons. The retention by cellophane of the antidiuretic substance in urine persisted whether or not the urine was first freed of protein by acid and heat. Walker (5) has called attention to differences in the permeability of various samples

TABLE II
The effect of dialysis on the antidiuretic activity of pituitrin and of human urine

Experiment number	Substance and dose	Treatment	Antidiuretic activity	Total 3-hour chloride excretion in the urine
84	Distilled water, 1.0 cc. per 100 grams of rat	Control	0	Microequivalents per 100 grams 34
	Pituitrin, 10 milliunits in 1.0 cc. distilled water per 100 grams of rat	Undialyzed	-7.0	239
		Dialyzed 3 hours	+2.1	71
18	Distilled water, 1.0 cc. per 100 grams of rat	Control	0	7
	Urine, hyperemesis gravidarum, 24 minutes' excretion in 1 cc. distilled water per 100 grams of rat	Dialyzed 3 hours	-5.5	6
		Dialyzed 6 hours	-6.2	14

of cellophane of the same thickness. It seems safe to conclude, however, that the antidiuretic substance of pituitrin passes through a cellophane membrane of suitable thickness far more easily than the antidiuretic substance found in human or rat urine.

4. *The effect of ultracentrifugation on the antidiuretic factor in pituitrin and in human urine*

An ultracentrifuge (23) with a 10° angle rotor (24) was used to compare the rate of sedimentation of these 2 antidiuretic substances. Seven samples of pituitrin in distilled water, in concentrations ranging from 2.75 to 27.5 milliunits per cc., were ultracentrifuged at 60,000 r.p.m. for periods of 4 to 6 hours. Eight samples of urine known to contain antidiuretic substance were treated in the same manner. At the end of the period of ultracentrifugation, the solutions were divided into 4 equal fractions numbering the uppermost, 1 and the lowermost, 4. Each fraction was assayed in the usual manner for its antidiuretic activity.

Figure 3 shows the results of a typical experiment. The antidiuretic activity of all 4 fractions of the pituitrin solution remained the same (Figure 3, upper) indicating that the antidiuretic substance of pituitrin had not been measurably concentrated by the ultracentrifuge. On the contrary, the upper 3 fractions of human urine became essentially inactive after ultracentrifugation while the fourth or lowermost fraction exerted an antidiuretic effect which was significantly greater than that of the original urine and far greater than that of the other 3 fractions of the same sample (Figure 3, lower).

Chloride excretion was high for all 4 fractions of the pituitrin solutions, corresponding in general magnitude to antidiuretic activity. Very slight concentration of pituitrin may have occurred in that the chloride concentration of the urine from the assay animals was 238 microequivalents per 100 grams for fraction 1 and 358 microequivalents per 100 grams for fraction 4. The antidiuretic substance of urine, however, had no effect on chloride excretion, even in fraction 4 which produced more antidiuresis than any of the fractions containing pituitrin in this comparison. Chloride

excretion was in the normal range and less than one-twentieth of that produced by pituitrin.

Figure 4 summarizes all of the results obtained. The grade of antidiuresis, expressed in sq. in. is indicated by the ordinate in the center of the chart. The activity of the top (1), middle (2 and 3) and bottom (4) fractions are indicated from left to right. Points referring to individual samples are connected by light lines while the heavy lines represent averages. The concentrations of the pituitrin solutions were varied to match the different intensities of antidiuretic activity exhibited by the samples of urine before ultracentrifugation.

With ultracentrifuged pituitrin, the variations in the 4 fractions of any 1 sample were within the admittedly large limits of experimental error in the assay method. No consistent difference existed, however, in the activity of the first and fourth fractions, as shown by the individual experiments and by the mean of 7 experiments. With ultracentrifuged urine, on the contrary (Figure 4, left), the fourth, or bottom, fraction in 8 individual experiments always had greater antidiuretic activity than the first, or top, fraction and the mean of these experiments slopes upward significantly for fraction 4. Moreover, in each experiment the antidiuretic activity of the fourth fraction was greater than that of the same urine before centrifugation. Prior removal of protein from urines containing small or large amounts did not alter the results.

In summary, the antidiuretic principle of commercial pituitrin cannot be concentrated by the ultracentrifuge, while the antidiuretic factor in urine can be concentrated readily by this method. The results agree with those to be expected from differences in dialyzability and suggest that the antidiuretic substance of urine either is, or is attached to, a larger molecule than the antidiuretic substance of commercial pituitrin.

5. *Properties of fresh extract and press juice of the posterior lobe of the pituitary gland with respect to dialysis and ultracentrifugation*

Rosenfeld (25) has called attention to certain differences between the physical properties of the "native" hormone of the pituitary gland and those of commercially purified pituitrin but he studied only the pressor and oxytocic fractions. It seemed

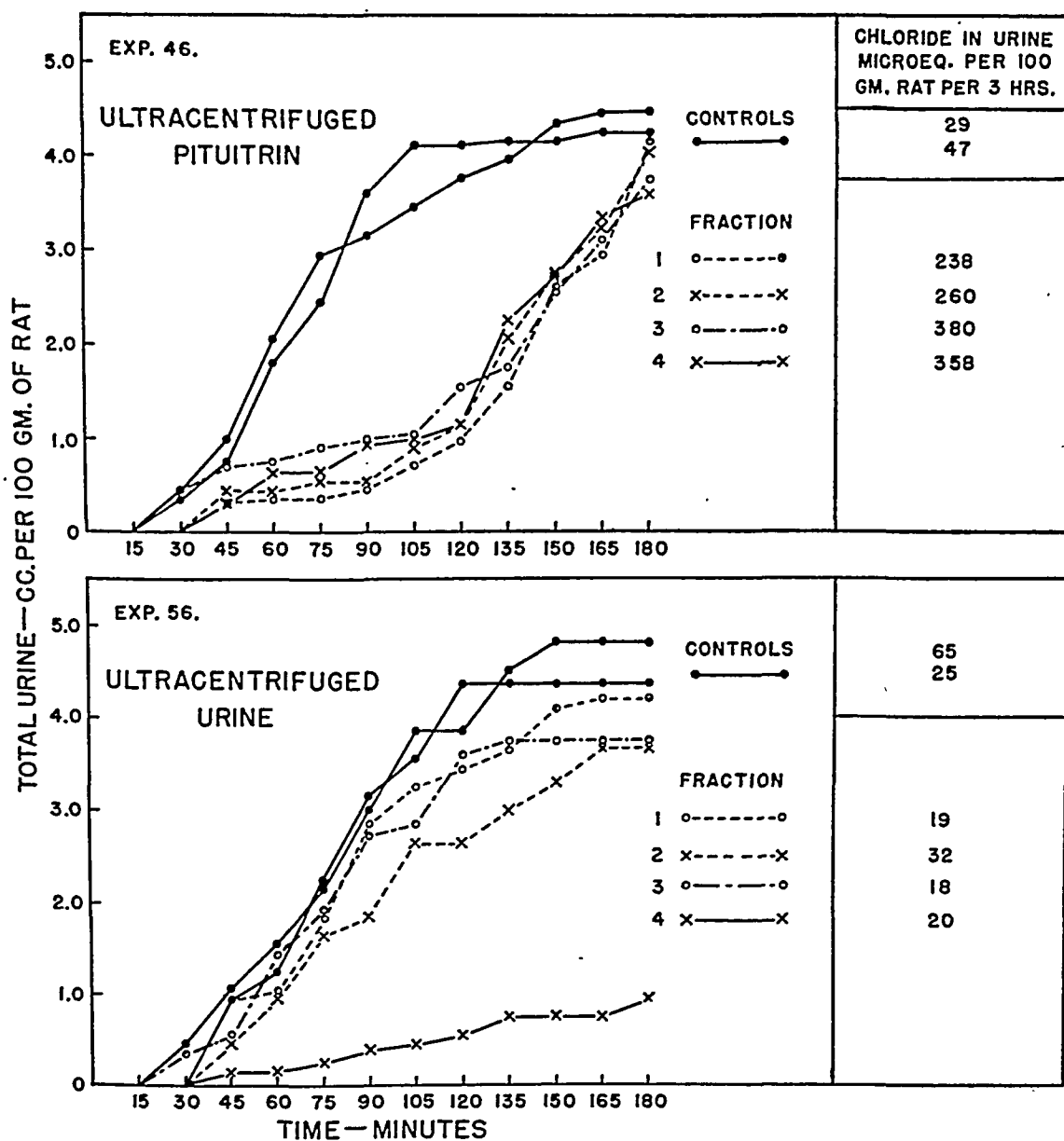


FIG. 3. UPPER: SHOWING THE ANTIDIURETIC ACTIVITY OF ULTRACENTRIFUGED PITUITRIN IN 4 FRACTIONS, NUMBERED 1 TO 4 FROM ABOVE DOWNWARD. LOWER: SHOWING THE ANTIDIURETIC ACTIVITY OF ULTRACENTRIFUGED POTENT URINE IN 4 FRACTIONS, SIMILARLY NUMBERED

desirable to extend these observations to antidiuretic and chloruretic activity of the "native" hormone, because pituitrin might not be wholly representative of the hormone as it exists in the body.

Saline extracts were prepared from the posterior lobes of beef pituitary glands, which had been kept solidly frozen since their removal from the

animals shortly after death. Five to 10 grams, wet weight, of material were alternately thawed at room temperature and frozen by dry ice 5 times. Sufficient 0.9 per cent NaCl solution at 5° C. was added to make a 10 per cent extract and the entire mixture was triturated in a porcelain mortar with finely ground glass until homogeneous. This red-brown solution was then filtered through

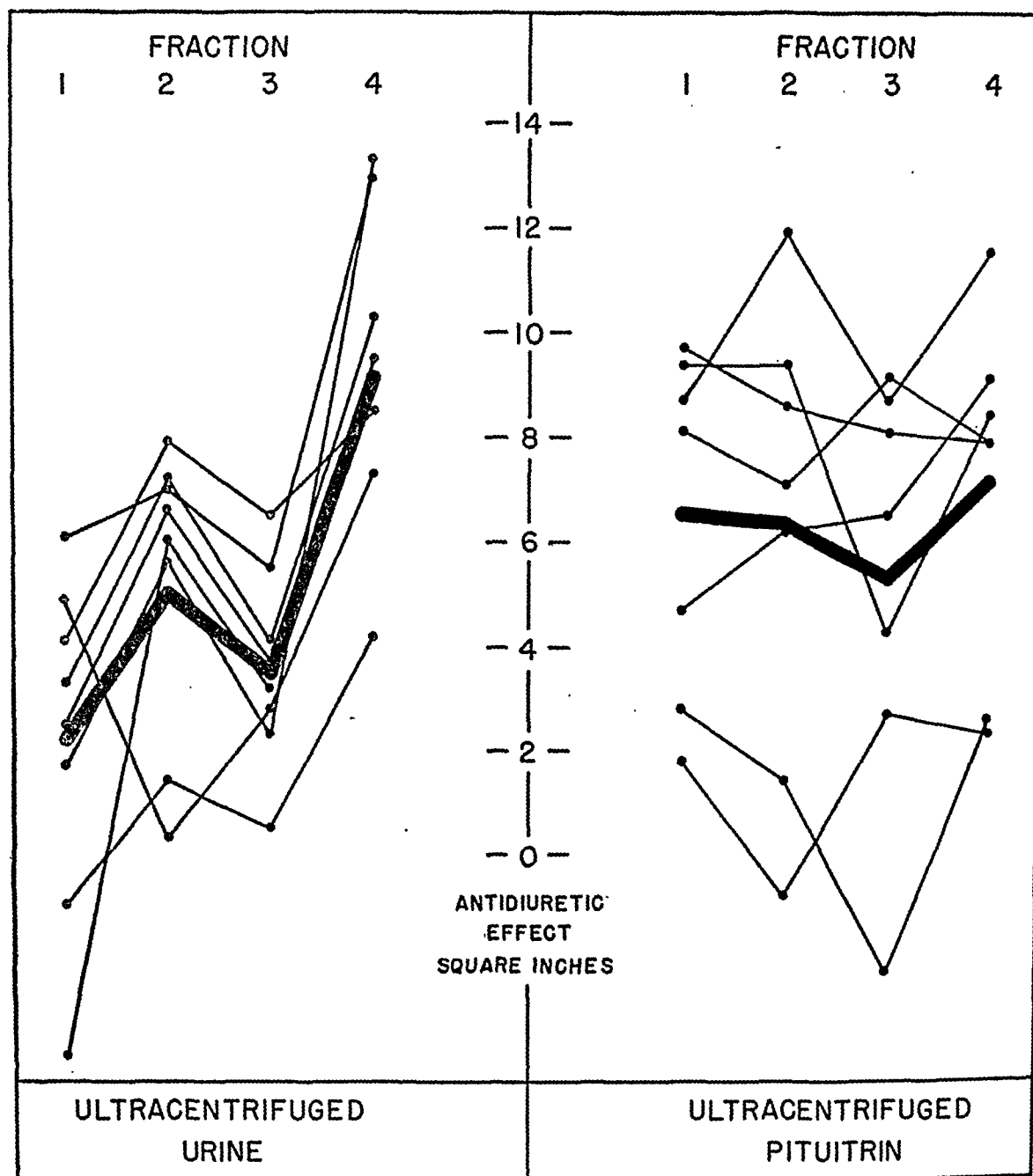


FIG. 4. SHOWING THE EFFECTS OF ULTRACENTRIFUGED PITUITRIN SOLUTIONS AND POTENT URINES (Fractions are numbered as in Figure 3)

Whatman No. 1 filter paper and stored at 5° C. The cloudy, opalescent filtrate was found to have marked antidiuretic and chloruretic activity. Several preliminary experiments indicated that the appropriate concentration for the rat assay method lay between 0.005 per cent and 0.05 per cent.

As shown in Table III, dialysis of these solutions in cellophane sacs for 3 hours reduced but did not abolish the antidiuretic and chloruretic activity of the original extract. Though the antidiuretic and chloruretic fractions in fresh extract are apparently dialyzable, it appears that for a

given membrane and a given period of oscillating dialysis, the antidiuretic fraction of commercial pituitrin passes through the membrane more rapidly than does the antidiuretic fraction from freshly extracted glands. Ultracentrifugation in 12 experiments on 4 different saline extracts showed that the antidiuretic and chloruretic activity of the bottom fraction was distinctly greater than that of the top fraction, as shown in Table III. It seems, therefore, that the antidiuretic and chloruretic fractions in fresh saline extract are attached to larger molecules, than is the case in

TABLE III

The effect of dialysis and ultracentrifugation on the antidiuretic and chloruretic activity of saline extracts of the posterior lobe of the pituitary gland

Ex-periment number	Substance and dose	Treatment	Antidiuretic activity	Total 3-hour chloride excretion in the urine
			sq. in.	Micro-equivalents per 100 grams
86	0.005 per cent Post. Pit. Extract No. 2	Undialyzed Dialyzed 3 hours	-6.8 -3.7	279 172
123	0.005 per cent Post. Pit. Extract No. 4	Ultracentrifuged 7 hours at 62,000 r.p.m.	-1.2 Top -8.2 Bottom	27 191
124	0.025 per cent Post. Pit. Extract No. 4	Ultracentrifuged 7 hours at 62,000 r.p.m.	-0.4 Top -4.6 Bottom	52 260

commercial pituitrin which has been treated by acid. At first sight it might appear impossible that the active substance of saline extract can be dialyzable, at least slowly and partially, and also be concentrated by the ultracentrifuge. Rosenfeld (25) has reported, however, that in untreated press juice of the posterior lobe of the pituitary, the pressor and oxytocic principles exist in the form of a single large molecule or 2 separate large molecules which by ultracentrifugation appear to have similar sedimentation rates. Because of the mild extraction method used, he believes these large molecules to be the "native" hormone. Rosenfeld showed further that pituitrin, pitressin and pitocin contained the active pressor and oxytocic principles in the form of physiologically effective cleavage products which have much smaller molecular weights than the "native" hormone.

This preponderance of "native" hormone of large molecular size in aqueous extract and press juice makes it possible to explain the apparent discrepancy mentioned above. Saline extract may initially contain "native" hormone for the most part with a small amount of the active cleavage product, the latter being dialyzable, the former undialyzable. It would then be expected that dialysis of the split hormone would lead slowly to further cleavage and more dialysis of antidiuretic substance. This process, continued long enough, would reduce the potency of the sample within the dialyzing sac but at a slower rate than would

the same dialysis of pure split products such as those found in commercial pituitrin. On the other hand, ultracentrifugation would not disturb the ratio of "native" and split hormone so that the active antidiuretic and chloruretic substance could be concentrated largely in the lowermost fraction.

To test this explanation, press juice was prepared by Dr. Morris Rosenfeld, ultracentrifuged, separated into 2 or 3 fractions, and then shipped to us for assays of antidiuretic and chloruretic activity. The top and bottom fractions of each sample were assayed in 12 experiments, using various concentrations in distilled water or 0.9 NaCl solution. Representative results are summarized in Table IV. In all experiments the antidiuretic and chloruretic factors were more concentrated in the lower fraction, though significant action was still perceptible in the top fraction in 2 of the experiments shown. In other respects also, as will be reported separately (22), the cleavage of the antidiuretic fraction in saline extracts resembles that of the pressor and oxytocic fractions of press juice.

In summary, the antidiuretic principle of the "native" hormone of the posterior pituitary gland still dialyzes but at a definitely slower rate than does the same principle in commercial pituitrin. Ultracentrifugation concentrates, at least partially, the antidiuretic principle of the "native" hormone, but does not concentrate that of pituitrin.

TABLE IV

The effect of ultracentrifugation on the antidiuretic and chloruretic activity of 4 different preparations of press juice

Ex-periment number	Substance and dose	Antidiuretic activity	Total 3-hour chloride excretion in the urine
		sq. in.	Micro-equivalents per 100 grams
95	0.1 cc. press juice No. 1 diluted to 80 cc. with distilled water 1.0 cc. per 100 grams of rat	-1.2 Top -4.8 Bottom	79 230
108	0.1 cc. press juice No. 2 diluted to 80 cc. with 0.9 per cent NaCl 1.0 cc. per 100 grams of rat	-4.9 Top -10.1 Bottom	68 218
114	0.1 cc. press juice No. 3 diluted to 120 cc. with 0.9 per cent NaCl 1.0 cc. per 100 grams of rat	-2.2 Top -7.9 Bottom	154 294
125	0.1 cc. press juice No. 4 diluted to 100 cc. with distilled water 1.0 cc. per 100 grams of rat	+0.60 Top -5.5 Bottom	37 161

6. Comparative effect of pituitrin, saline pituitary extract, pituitary press juice, and the antidiuretic substance of urine, on excretion of the chloride in the urine (chloruresis)

It is well known that pituitrin, in addition to its pressor, oxytocic, and antidiuretic effects, also increases the excretion of chloride in the urine. Even during marked antidiuresis the total excretion of chloride over a given time exceeds many-fold the normal total excretion of chloride. The concentration of chloride per unit volume of urine shows, of course, even more striking increase. Because pituitrin, so far as is known at present, is the only substance which produces simultaneous antidiuresis and chloruresis, it seemed desirable to

study the action of the antidiuretic substance of urine in this respect.

The total chloride excreted during 3 hours by each group of assay rats, is shown in Figure 5. Total chloride excretion, in terms of microequivalents per 100 grams of rat in 3 hours, is shown on the ordinate and plotted against the antidiuretic activity of the same sample of urine, in sq. in., on the abscissa. The dotted line, just below 90 microequivalents, indicates the maximal chloride excretion observed in animals receiving control injections. For the sake of clearness, individual control values are not shown. Pituitrin (open circles), crude extracts of fresh pituitary glands (crosses), and press juice of fresh pituitary gland

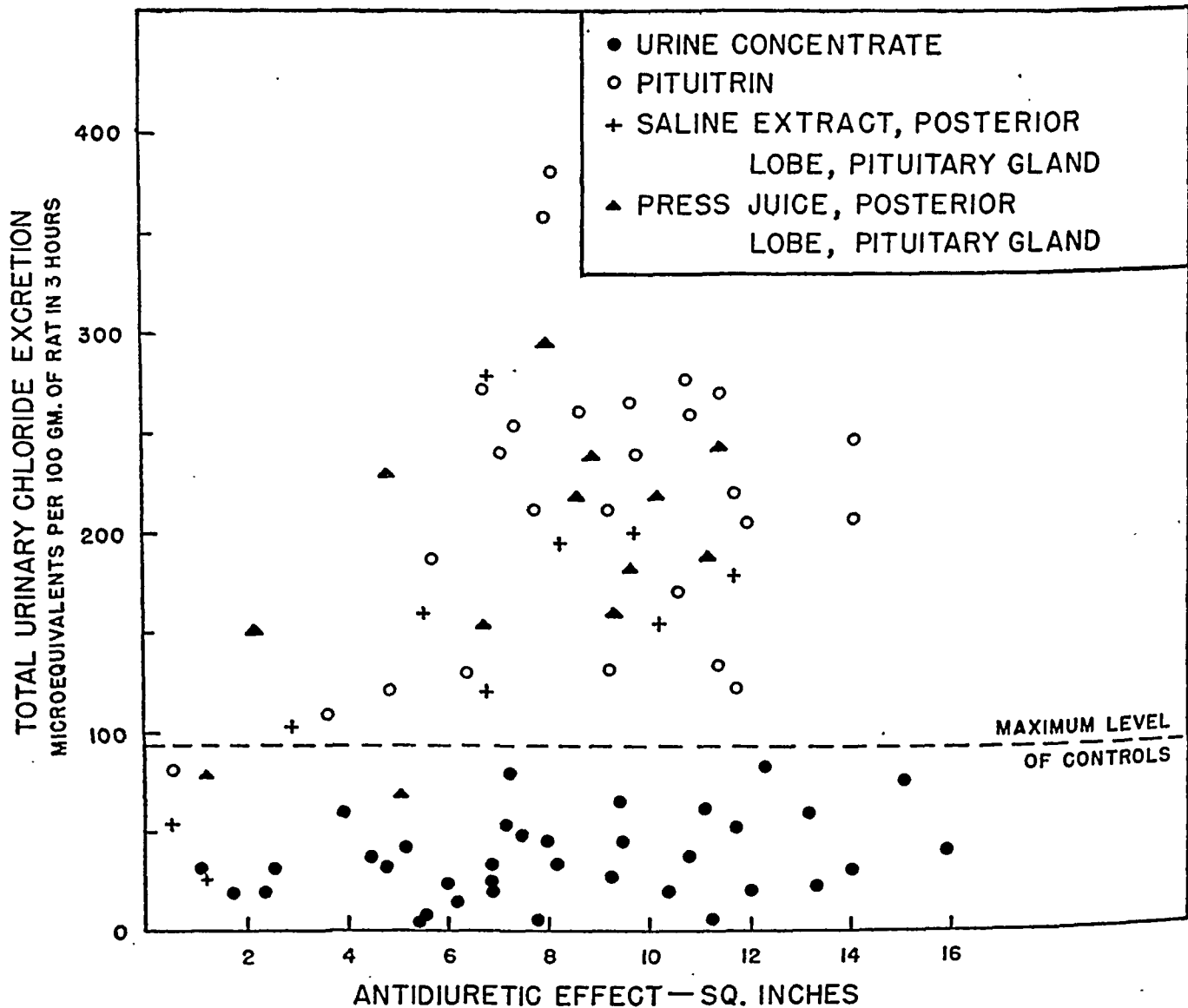


FIG. 5. SHOWING THE RELATION BETWEEN THE ANTIDIURETIC AND CHLORURETIC ACTIVITY OF URINE CONCENTRATES (DOTS), PITUITRIN (CIRCLES), SALINE EXTRACT OF THE POSTERIOR LOBE OF THE PITUITARY GLAND (CROSSES), AND PRESS JUICE OF THE POSTERIOR LOBE OF THE PITUITARY GLAND (TRIANGLES)

(solid triangles) increased the excretion of chloride roughly in proportion to the grade of antidiuresis. Total chloride excretion by the assay animals receiving pituitary products was above the maximal level of the control experiments, except for 5 instances in which the dose of pituitary products was so small that the antidiuretic effect was barely detectable. Thus the "native" hormone and its derivative, pituitrin, were chloruretic as well as antidiuretic and this relation held, irrespective of differences in dialyzability or molecular size. There was no evidence that the antidiuretic and chloruretic effects could be separated.

In striking contrast dialyzed and concentrated urine (solid dots, Figure 4), even when conspicuously antidiuretic, never increased chloride excretion above the level of the controls.

7. The antidiuretic activity of placental extracts

The observations so far described suggest that the antidiuretic substance of urine differs in certain important respects from the antidiuretic principle of the pituitary gland and may conceivably arise elsewhere as suggested by Walker (5). If this be true, other sources of antidiuretic material must be considered. Because conspicuous diuresis usually follows parturition in patients with toxemia of pregnancy, it seemed logical to investigate the placenta first. Saline extracts of 11 fresh placentas were studied, 6 from women delivered after normal pregnancy and 5 from women delivered in the course of a toxemia of pregnancy.

The freshly delivered placentas were taken immediately to the laboratory. The cord and membranes were carefully dissected away and the surface of the placenta was gently washed free of blood clots. A careful examination of the surface for fresh or old infarcts was followed by multiple sectioning and observation of the cut surface. The whole placenta was then cut into small pieces with scissors and passed through a coarse meat grinder and weighed. An equal amount of 0.9 NaCl solution was then added and the entire mixture was ground at a temperature not over 20° C. in an Eppenbach colloid mill until completely homogeneous. This mixture was filtered through 3 thicknesses of surgical gauze and the filtrate centrifuged. The supernatant fluid was

decanted and dialyzed in cellophane tubes against running tap water for 3 hours on an oscillating platform. The dialyzed material was stored at 5° C. and bio-assay completed the next day.

Figure 6 compares the results observed with an extract of normal placenta (above) and a placenta from a patient with toxemia (below). The 50 per cent extract of normal placenta was inactive; the same was true of a 250 per cent extract concentrated by evaporation from the frozen state and redissolved in one-fifth the original volume of water. In contrast, 50 and 25 per cent extracts of the placenta from a patient with toxemia of pregnancy were markedly and moderately antidiuretic respectively.

The antidiuretic activities of all the extracts studied are summarized in Table V. With 1 notable exception (experiment 69), the extracts of all normal placentas were slightly diuretic, inactive, or slightly antidiuretic. No logical explanation can be offered for the moderate activity observed in experiment 69. The patient was a 21-year-old colored primigravida in good health, without edema, albuminuria, or hypertension. She had a normal and uneventful delivery and puerperium. It is conceivable that changes in the placenta had occurred too recently in this case to produce systemic effect.

Extracts of placentas from patients with toxemia of pregnancy were moderately to markedly antidiuretic, again with one notable exception, experiment 112, in which the extract was only

TABLE V

Comparison of the antidiuretic activity of 50 per cent extracts prepared from placentas obtained from patients with normal pregnancy and with toxemia of pregnancy

Normal subjects		Patients with toxemia of pregnancy	
Experiment number	Antidiuretic activity	Experiment number	Antidiuretic activity
	<i>sq. in.</i>		<i>sq. in.</i>
62	-0.7	55	-7.3
71	-4.0	68	-12.6
106	+0.7	75	-8.0
104	+1.7	112	-3.9
107	-2.4	63	-6.4*
69	-6.9		
Average	-1.9	Average	-7.6

* The antidiuretic activity of this extract after dialysis for an additional 3 hours was 6.0 sq. in.

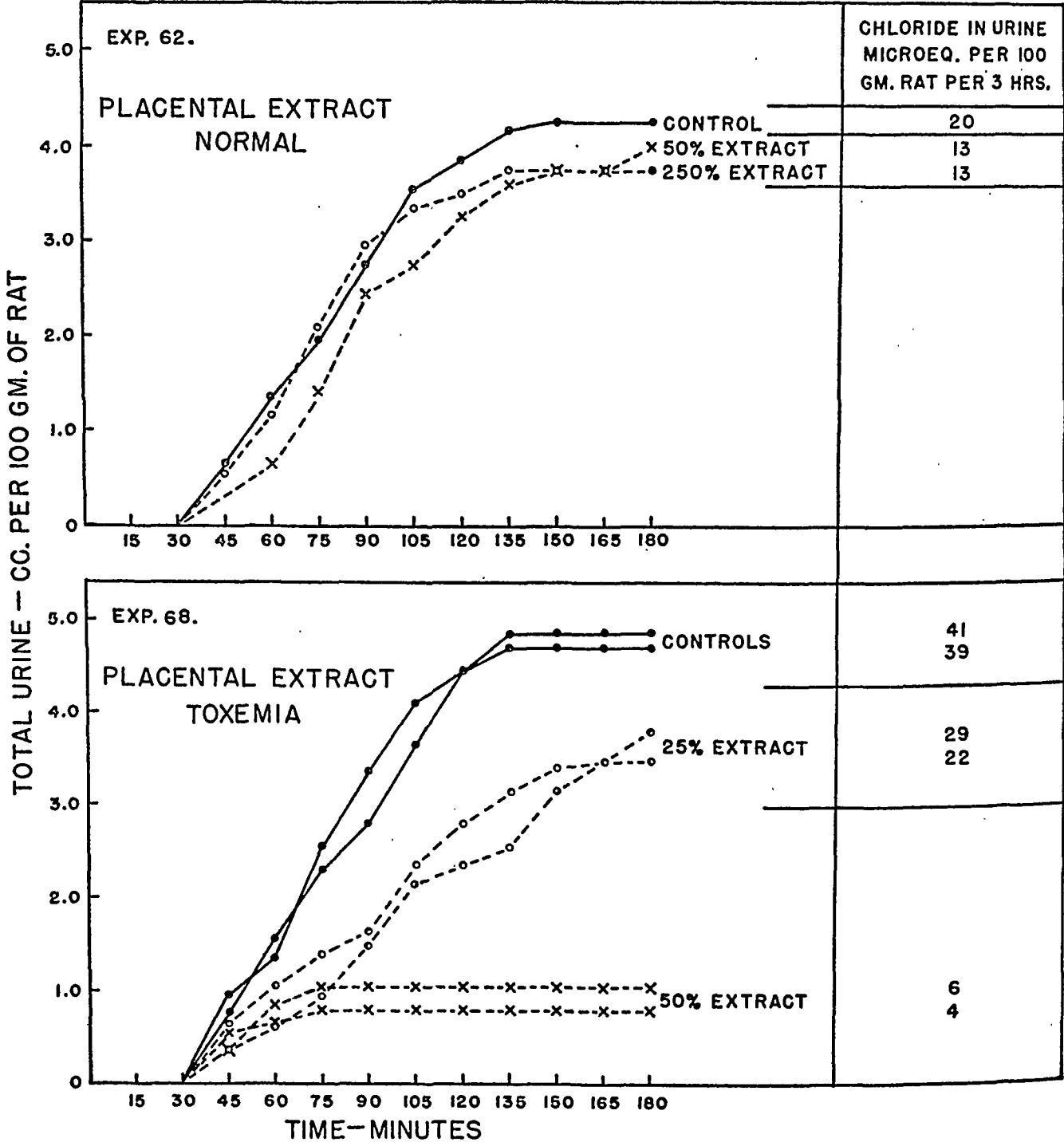


FIG. 6. UPPER: SHOWING THE RESULTS OF ASSAY OF 50 PER CENT AND 250 PER CENT EXTRACTS OF A NORMAL PLACENTA. LOWER: SHOWING THE RESULTS OF ASSAY OF 25 PER CENT AND 50 PER CENT EXTRACTS OF A PLACENTA FROM A PATIENT WITH TOXEMIA OF PREGNANCY

slightly antidiuretic. This patient, a 24-year-old colored primigravida, had a blood pressure of 220 mm. Hg systolic and 140 mm. Hg diastolic, bilateral choked discs, and retinal separation but no demonstrable edema. The other patients with toxemia all presented clinical edema with significant albuminuria and hypertension. Despite considerable variation, the average figures in Table V

indicate that placentas from patients with toxemia contained considerably more antidiuretic substance than did placentas obtained after normal pregnancies.

The properties of the antidiuretic substance in placental extracts resembled those of the antidiuretic substance in the urine of these patients. (a) As shown in experiment 63 of Table V,

dialysis for an additional 3 hours did not reduce the antidiuretic activity of the extract. (b) Ultracentrifugation of the extract of experiment 55 for 4 hours concentrated the antidiuretic substance in the lower fraction as shown in Figure 7. (c) Chloride excretion by the assay animals was tested in each instance and was never increased even during marked antidiuresis as shown in Figure 7 to the right.

While these results indicate that in the pregnant woman the placenta may contain antidiuretic material, still other sources must be considered to explain the excretion of antidiuretic substance by dehydrated male rats, male human subjects (8) and dehydrated non-pregnant women. Theobald and White (26), Walker (27) and Schaffer *et al.* (28) have reported that certain liver extracts are antidiuretic. Preliminary studies of our own have indicated also that crude 50 per cent saline extracts of rat livers are moderately antidiuretic but still have no effect on chloride excretion. A 50 per cent extract of abdominal muscle from the rat did not affect the excretion of either water or chloride.

With respect to extracts of kidney tissue, results are more complicated. Pickering and Prinzmetal (29) reported that rabbit kidney extract was

definitely antidiuretic when injected into the dog but the same extract injected into the rabbit produced transient antidiuresis followed by copious diuresis and simultaneous increase in the excretion of both sodium and chloride. The diuresis and chloruresis were ascribed to renin, the antidiuresis was not studied.

Using a previously described method (30), 9 extracts were prepared from the normal kidneys of rabbits and rats. While antidiuretic activity was observed with certain concentrations and certain routes of injection, the results were complicated by the simultaneous or subsequent diuretic effect of renin. Of the tissues so far explored, nothing can be reported except that extracts of normal liver and kidney are probably moderately antidiuretic under certain conditions and that extracts of muscle are inactive. Further study is obviously necessary before conclusions can be drawn.

DISCUSSION

Evidence for (1 to 3, 6) and against (4, 5, 27) the thesis that the antidiuretic substance of urine arises in the pituitary glands has already been described. The results of comparing the anti-

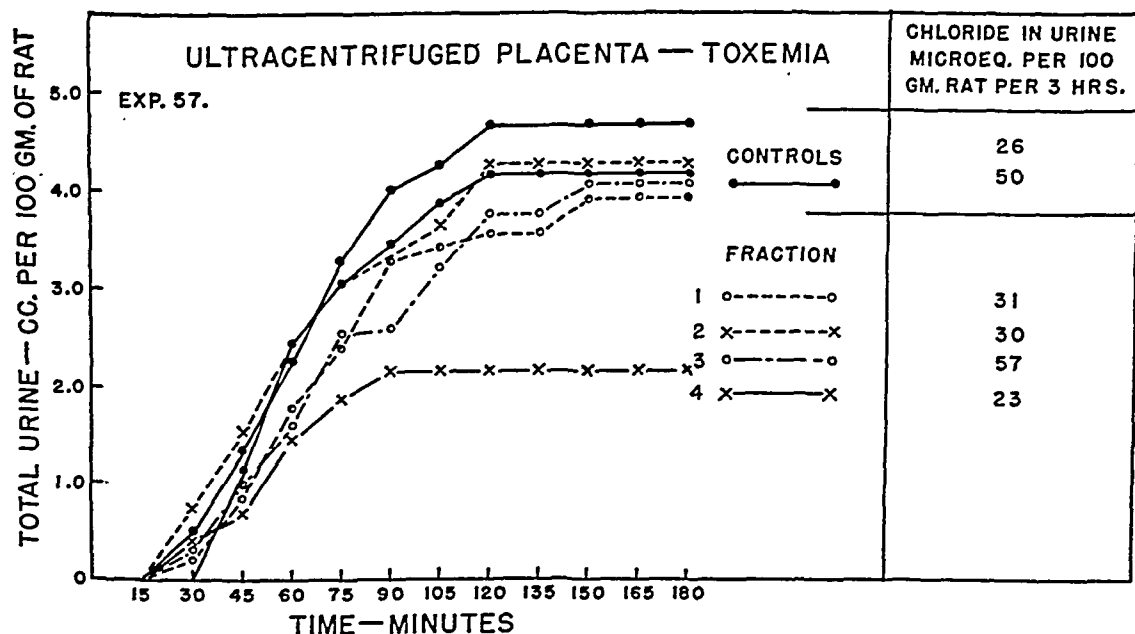


FIG. 7. SHOWING THE EFFECT OF ULTRACENTRIFUGATION (4 HOURS AT 60,000 R.P.M.) ON THE ANTIDIURETIC AND CHLORURETIC ACTIVITY OF PLACENTAL EXTRACT (Fractions numbered 1 to 4 from above downward)

TABLE VI

Comparison of the antidiuretic substance in urine and in placental extracts with the antidiuretic substance of the pituitary gland prepared by 3 methods

Source of antidiuretic substance	Excretion of chlorides in urine	Effect of ultracentrifugation	Passage through cellophane
Urine.....	No effect	Concentrated	Not dialyzable
Placental extract....	No effect	Concentrated	Not dialyzable
Commercial pituitrin	Increased	Not concentrated	Dialyzable (rapid)
Saline extracts of posterior lobes of pituitary glands.....	Increased	Concentrated	Dialyzable (slow)
Press juice of posterior lobes of pituitary gland.....	Increased	Concentrated	?

diuretic substance in urine and placenta with the better known antidiuretic substance of the pituitary gland are summarized in Table VI. Though similar in reducing the excretion of water, these two substances differ most strikingly with relation to their effects on chloride excretion. The pituitary substance, prepared by three methods and administered in adequate dosage, always increased chloride excretion even when antidiuretic activity was barely detectable. The antidiuretic substance of urine always failed to affect chloride excretion, even when conspicuous antidiuresis was produced.

Ultracentrifugation and dialysis also demonstrated clear cut differences between commercial pituitrin and the antidiuretic substance of urine, but when more "natural" preparations of the posterior lobe of the pituitary gland were studied, these differences became relative rather than absolute. It is unfortunate that freshly prepared press juice was not available for dialysis but the results nevertheless indicate that the antidiuretic substance of pituitary gland in its "native" state is probably attached loosely to a protein. Its physical properties resemble to this extent those of the pressor and oxytocic substances of the pituitary gland as described by Rosenfeld (25).

It is conceivable that the urinary factor is concentrated by the ultracentrifuge and is undialyzable merely because a small molecule arising in the pituitary gland has, in the course of transportation and excretion, become attached to a large molecule. However, this does not explain the observation that the urinary substance does not affect chloride excretion. If the antidiuretic substance of urine actually originates in the pituitary gland it is necessary to postulate, in addition, a splitting of the chloruretic and the antidiuretic factors, with de-

struction or neutralization of the former somewhere in the body. Separation of these factors has not yet been accomplished *in vitro*. This possibility *in vivo* is now being tested further in patients with diabetes insipidus by studying the antidiuretic and chloruretic activity of their urine before and after the administration of pitressin in moderate and large doses.

Another possibility is that the antidiuretic substance of urine originates in some organ or tissue other than the pituitary gland, and never did possess chloruretic activity. This explanation is supported by the presence, in extracts of placentas from patients with toxemia of pregnancy, of an antidiuretic substance which resembled, in the three respects tested, the antidiuretic factor found in the urine of these patients. Whether the placenta merely stored this antidiuretic substance, or actually produced it, cannot be stated at this time. The placenta is certainly not the only source of antidiuretic substance, because normal men and non-pregnant women excrete this factor in their urine if they are sufficiently dehydrated. Other possible sources are liver (27) and kidney (29). Extracts of liver, while antidiuretic, had no effect on chloride excretion, whereas kidney extract produced various effects consisting of mixtures of antidiuresis without effect on chloride excretion, and profound diuresis with marked increase in chloride excretion (29). Pickering and Prinzmetal believe diuresis and chloruresis to be the effect of renin. Injection of human kidney extracts into rabbits has demonstrated that antidiuresis is usually produced without affecting chloride excretion; in a few instances these extracts produced diuresis along with increase in chloride excretion (31) but these effects bore no relation to the existence of hypertension during life. No conclusion is warranted except that further studies of kidney extracts are needed.

The edema observed in cases of toxemia of pregnancy may be associated with a low plasma protein percentage, but in other instances cannot be adequately explained by the physical factors included in the Starling hypothesis. Teel and Reid (6) expressed the opinion that the antidiuretic substance found in the urine of patients with eclampsia could be related to water retention but not to the grade of hypertension. The importance of gross pathological changes in the

placentas of toxemic patients has been suggested (32) but is not generally accepted (33). Weiss *et al.* (34) concluded that "the presence of a functioning placenta maintained the syndrome and removal of the placenta is responsible for improvement," but were unable to demonstrate any pressor factor in the placentas of patients with toxemia of pregnancy. Of considerable interest, therefore, are the findings (a) that antidiuretic substance was found in urine of a patient with toxemia and hydatid mole, and (b) that greater amounts of antidiuretic substance are found in the placentas of patients with toxemia of pregnancy than in those with normal pregnancies. It has also been found (6, 7) that after delivery the antidiuretic substance disappears rapidly from the urine of patients with toxemia of pregnancy as diuresis sets in.

To assay accurately the antidiuretic activity of urine may be more difficult than heretofore realized. Noble *et al.* (35) have reported that the urine contains augmentor substances which can produce erroneously high values for antidiuretic activity when pituitary extracts are injected subcutaneously. Schaffer *et al.* (28), using Walker's method (5), of collodion adsorption, reported that lower values of antidiuretic activity were obtained by the adsorption method than were found following dialysis of the same urine. It has often been assumed that the antidiuretic substance in urine arises in the pituitary gland and acts like pituitrin, but it has not been established that these properties apply also to the antidiuretic substance in urine. In agreement with Schaffer *et al.* (28); the present results indicate that in quantitative assays it would be best to compare the yield of the dialysis and adsorption methods with known amounts of urinary antidiuretic substance before far-reaching conclusions are drawn.

CONCLUSIONS

In agreement with previous observations, it was found that the urine from patients with toxemia of pregnancy contained large amounts of antidiuretic substance. Patients with hydatid mole (1 case) and hyperemesis gravidarum (3 cases) were also found to excrete significant amounts of antidiuretic substance.

Whereas commercial pituitrin was dialyzable through cellophane, was not concentrated by the ultracentrifuge, and increased chloride excretion in the urine, the antidiuretic substance in urine did not pass through cellophane, was concentrated by the ultracentrifuge, and did not affect chloride excretion in the urine.

The results so far indicate that an antidiuretic substance, differing in important respects from the hormone of the posterior pituitary, can appear in the urine of human beings.

Studies on fresh saline extracts and on press juice of the posterior lobe of the pituitary gland indicated that the antidiuretic and chloruretic factor of the pituitary dialyzed more slowly than did commercial pituitrin and could be concentrated by the ultracentrifuge. In this more "natural" state, however, the pituitary substance still retained quantitatively its effect upon chloride excretion, and still differed radically in this respect from the antidiuretic substance found in urine.

The placentas of patients with toxemia of pregnancy contained larger quantities of antidiuretic substance than did placentas from normal patients. The antidiuretic substance from placentas resembled in all three respects the antidiuretic substance found in urine of such patients. The liver and kidney are considered briefly as other possible sources of antidiuretic activity.

We wish to thank Dr. Alfred Chanutin for the use of the ultracentrifuge and Dr. Morris Rosenfeld for his generous supply of press juice. We are indebted to Mrs. Carolyn Carr and Mr. Herman Goslyn for technical assistance.

BIBLIOGRAPHY

1. Gilman, A., and Goodman, L., The secretory response of the posterior pituitary to the need for water conservation. *J. Physiol.*, 1937, 90, 113.
2. Ingram, W. R., Ladd, L., and Benbow, J. T., The excretion of antidiuretic substance and its relation to the hypothalamico-hypophyseal system in cats. *Am. J. Physiol.*, 1939, 127, 544.
3. Martin, S. J., Herrlich, H. C., and Fazekas, J. F., Relation between electrolyte imbalance and excretion of an antidiuretic substance in adrenalectomized cats. *Am. J. Physiol.*, 1939, 127, 51.
4. Arnold, O., Über blutdrucksteigernde und diuresehemmende Substanzen im Harn. *Arch. f. exper. Path. u. Pharmacol.*, 1938, 190, 360.
5. Walker, A. M., Experiments upon the relation between the pituitary gland and water diuresis. *Am. J. Physiol.*, 1939, 127, 519.

6. Teel, H. M., and Reid, D. E., Observations upon the occurrence of an antidiuretic substance in the urine of patients with preeclampsia and eclampsia. *Endocrinology*, 1939, 24, 297.
7. Krieger, V. I., and Kilvington, T. B., Antidiuretic substance in urine in relation to normal and toxemic pregnancy. *M. J. Australia*, 1940, 1, 575.
8. Robinson, F. H., Jr., and Farr, L. E., The relation between clinical edema and the excretion of an antidiuretic substance in the urine. *Ann. Int. Med.*, 1940, 14, 42.
9. Anselmino, K. J., Hoffman, F., and Kennedy, W. P., The relation of hyperfunction of the posterior lobe of the hypophysis to eclampsia and the nephropathy of pregnancy. *Edinburgh M. J.*, 1932, 39, 376.
10. Theobald, G. W., The alleged relation of hyperfunction of the posterior lobe of the hypophysis to eclampsia and the nephropathy of pregnancy. *Clin. Sc.*, 1934, 1, 225.
11. de Wesselow, O. L. V. S., and Griffiths, W. J., On the question of pressor bodies in the blood of hypertensive subjects. *Brit. J. Exper. Path.*, 1934, 15, 45.
12. Hurwitz, D., and Bullock, L. T., Failure to find pressor and antidiuretic substances in patients with toxemia of pregnancy. *Am. J. M. Sc.*, 1935, 189, 613.
13. Byrom, F. B., and Wilson, C., The alleged pituitary origin of eclamptic and preeclamptic "toxaemias" of pregnancy. *Quart. J. Med.*, 1934, 3, 361.
14. Levitt, G., The problem of an antidiuretic substance in the blood of patients with eclampsia and other hypertensive diseases, with observations on spinal fluid. *J. Clin. Invest.*, 1936, 15, 135.
15. Melville, K. I., Antidiuretic pituitary substance in blood, with special reference to the toxemia of pregnancy. *J. Exper. Med.*, 1937, 65, 415.
16. Marx, H., and Schneider, K., Untersuchungen zur Diurese; Über den Nachweis antidiuretischer Substanzen im Blute. *Arch. f. exper. Path. u. Pharmacol.*, 1934, 176, 24.
17. Burn, J. H., The estimation of the antidiuretic potency of pituitary (post. lobe) extracts. *Quart. Jour. Pharm. and Pharmacol.*, 1931, 4, 517.
18. Silvette, H., The influence of post-pituitary extract on the excretion of water and chlorides by the renal tubules. *Am. J. Physiol.*, 1940, 128, 747.
19. Ham, G. C. (To be published.)
20. Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry. *Methods*. Williams & Wilkins, Baltimore, 1932, p. 835.
21. Silvette, H., Effect of diminishing doses of post-pituitary extract on urinary excretion of water and chlorides. *Proc. Soc. Exper. Biol. and Med.*, 1940, 45, 599.
22. Ham, G., and Rosenfeld, M., Ultracentrifugation of the antidiuretic, chloruretic, and pressor factors of posterior pituitary extracts. (To be published in *Bull. Johns Hopkins Hosp.*)
23. Beams, J. W., Linke, F., and Sommer, P. H., A vacuum type air-driven ultracentrifuge. *Rev. Scient. Instruments*, 1938, 9, 248.
24. Masket, A. V., A quantity type rotor for the ultracentrifuge. *Rev. Scient. Instruments*, 1941, 12, 277.
25. Rosenfeld, M., The native hormones of the posterior pituitary gland: The pressor and oxytocic principles. *Bull. Johns Hopkins Hosp.*, 1940, 66, 398.
26. Theobald, G. W., and White, M., An antidiuretic substance extracted from the liver. *J. Physiol.*, 1933, 78, 18 p.
27. Walker, A. M., An antidiuretic effect produced by certain preparations of heparin. *Proc. Soc. Exper. Biol. and Med.*, 1938, 39, 105.
28. Schaffer, N. K., Cadden, J. F., and Stander, H. J., Measurement of antidiuretic activity as applied to eclamptic urine and properties of antidiuretic substance in rat urine, pituitary and beef liver. *Endocrinology*, 1941, 28, 701.
29. Pickering, G. W., and Prinzmetal, M., The effect of renin on urine formation. *J. Physiol.*, 1940, 98, 314.
30. Landis, E. M., Jeffers, W. A., and Shiels, E. H., The pressor effects of homologous and heterologous injections of heated kidney extracts. *Am. J. Physiol.*, 1940, 128, 672.
31. Landis, E. M., Unpublished observations.
32. Bartholomew, R. A., Pathology of the placenta with special reference to infarcts and their relation to toxemia of pregnancy. *J. A. M. A.*, 1938, 111, 2276.
33. Harer, W. B., A study of one thousand placentas. *Am. J. Obst. and Gynec.*, 1936, 32, 794.
34. Weiss, S., Dexter, L., Parker, F., Jr., and Tenney, B., Jr., Arterial hypertension in pregnancy and the hypertensive toxemia syndrome of pregnancy (preeclampsia and eclampsia). *Tr. A. Am. Physicians*, 1940, 55, 282.
35. Noble, R. L., Rinderknecht, H., and Williams, P. C., The apparent augmentation of pituitary antidiuretic action by various retarding substances. *J. Physiol.*, 1939, 96, 293.

STUDIES WITH RADIOACTIVE DI-AZO DYES. 1. THE LOCALIZATION OF RADIOACTIVE DI-BROM TRYPAN BLUE IN INFLAMMATORY LESIONS

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The localization of circulating colloidal dyes in areas of increased capillary permeability, and their ingestion there by phagocytic cells, has been under investigation for the past 30 years. First observed by Goldmann (1) during his early studies with the vital stains, it later became the subject of study by investigators working with the inflammatory process, who found that many types of inflammation caused sufficient increase in permeability of the capillaries to produce local accumulations of dye (2, 3, 4).

Evans (5) described the macrophage system as a separate entity as a result of its delineation by such dyes, following an extensive study of the intra-vitam reactions of many dyes of the benzidine series, such as trypan blue (6). More recently, many workers (7 to 10) have used dye-accumulation as an index of the extent of permeability changes following injections of various irritants, fractions of inflammatory exudates, or organ-extracts, in an effort to elucidate the details of the inflammatory sequence. Burrows (11) provides an excellent review of the work in this field prior to 1932.

Keith, Rowntree and Geraghty (12) explored the use of colloidal dyes in blood-volume determinations, this application arising from the fact that many of the acid azo dyes remain intravascular for fairly prolonged periods of time, and appear to form a bond with plasma protein (13). They, and others who followed in the same field (Dawson, Evans and Whipple (14), and Gregeron, Gibson and Stead (15)) have added much to our knowledge of the intravascular reactions of the dyes through their quantitative observations on serum concentrations, rates of elimination from the blood stream, and toxicity. Through this

work a new dye came into common use, called Evans blue (so named because its biological properties were first described by Prof. H. M. Evans (14)), otherwise known as T-1824. The latter name is based on the structure of the dye, indicating that tolidine is coupled with the "1-8-2-4" acid (1-amino-8-naphthol-2, 4-di-sulfonic acid). This dye is isomeric with trypan blue, which by the same type of nomenclature would be "T-1836."

In the past five years, work has begun to appear in the literature bearing on the potential clinical application of the property of these dyes of concentrating in abscesses. Menkin (9) saw the potentialities of this field and wrote that trypan blue might be used to enhance the roentgen appearance of abscesses. Strauss, *et al.* (16) conceived the idea that some substance which would concentrate in abscesses might be made radioactive and so be useful as a means of localizing them clinically. This group has studied various radioactive and non-radioactive substances including a non-radioactive brominated Evans blue, and a radioactive brominated "H-acid" (the 1-8-3-6 sulfonic acid comprising a portion of the trypan blue molecule) (17, 18). They did not employ very large doses of radioactivity and their radioactive readings from intact animals were quite low. Work is not reported with an actual radioactive dye; H-acid is neither colloidal nor a dye, although it is a dye-intermediate used in the manufacture of trypan blue. They conclude that the method holds some promise and deserves further work.

A colloidal dye such as trypan blue, by virtue of its property of accumulation in abscesses, offers us a means of diagnosing localized inflammation if we can make such a dye radioactive. The dye must be radioactive to a degree permitting its detection from outside the patient with a Geiger counter. Furthermore, the radioactive element must be firmly affixed to the molecule by a non-

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dissociating bond so that the radioactivity is carried throughout its course in the body by the larger organic molecule. For example, a sodium salt of such a dye made with radioactive sodium would be useless for such a purpose because the sodium is attached to the molecule by a polar bond, and dissociates in solution. Therefore, the radioactive sodium would exchange with stable sodium immediately upon introduction into the circulation and the dye would no longer, in any sense, be radioactive.

Were this diagnostic tool to be perfected, lesions such as appendiceal, sub-diaphragmatic or brain abscesses would become more certain of diagnosis and more susceptible to accurate treatment.

We wish herein to report the study of the localization in inflammatory lesions of a radioactive colloidal di-azo acid dye, di-brom trypan blue.

EXPERIMENTAL PROCEDURE

1. Chemistry

The trypan blue molecule consists of a di-phenyl diamine (ortho-tolidine) coupled through two azo linkages

to two equivalents of H-acid (1-amino-8-naphthol-3, 6-disulfonic acid). We have rendered the molecule radioactive by adding two atoms of radio-bromine to the ortho-tolidine, and then coupling it with the acid to make the finished dye. The structural formula of the dye and the position of the bromines is shown in Figure 1.

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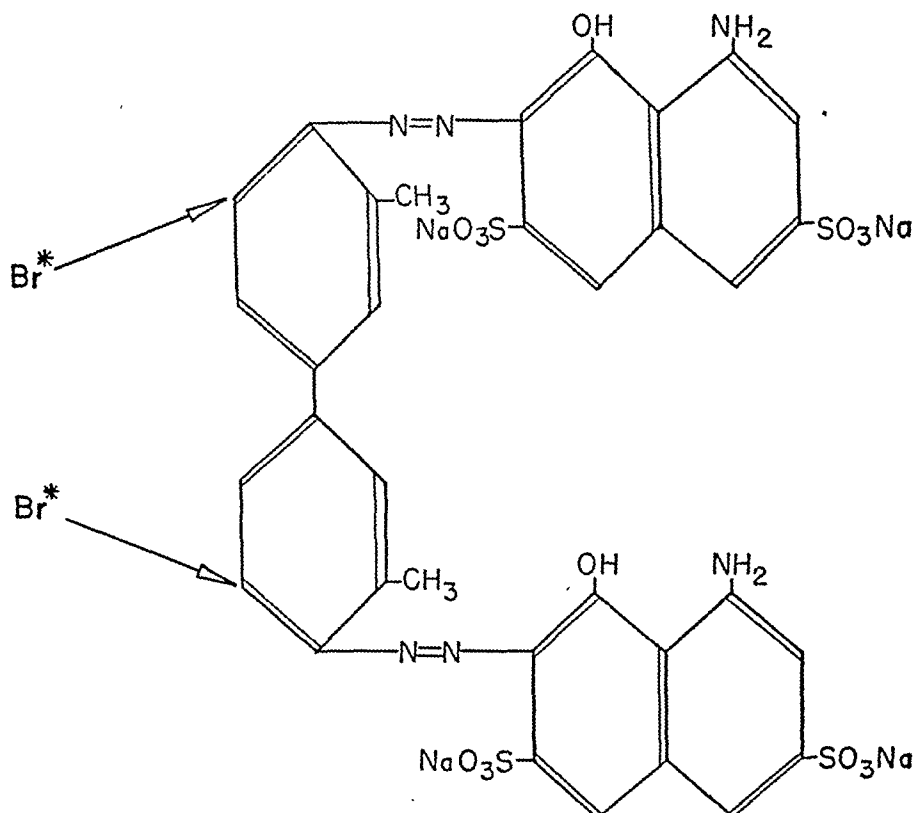


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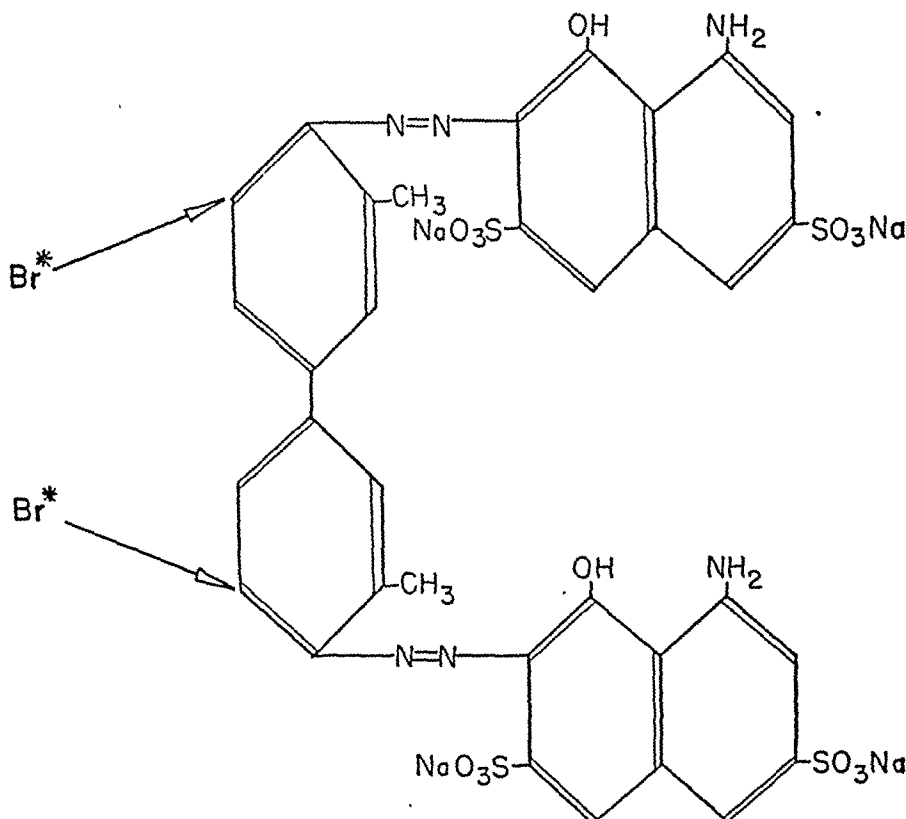


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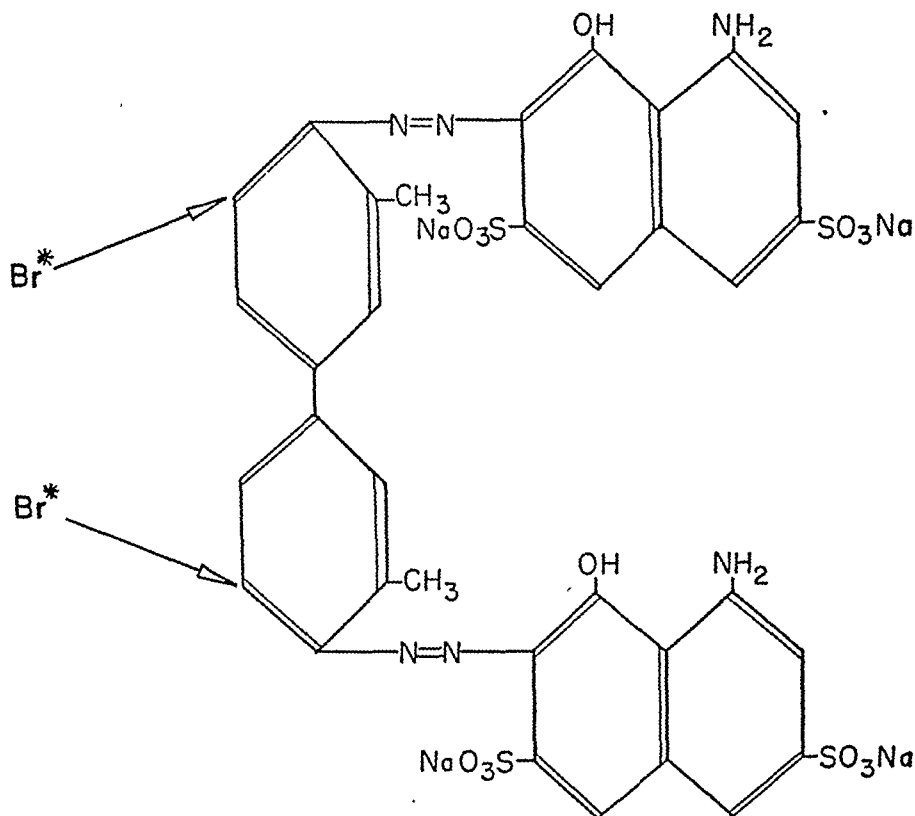


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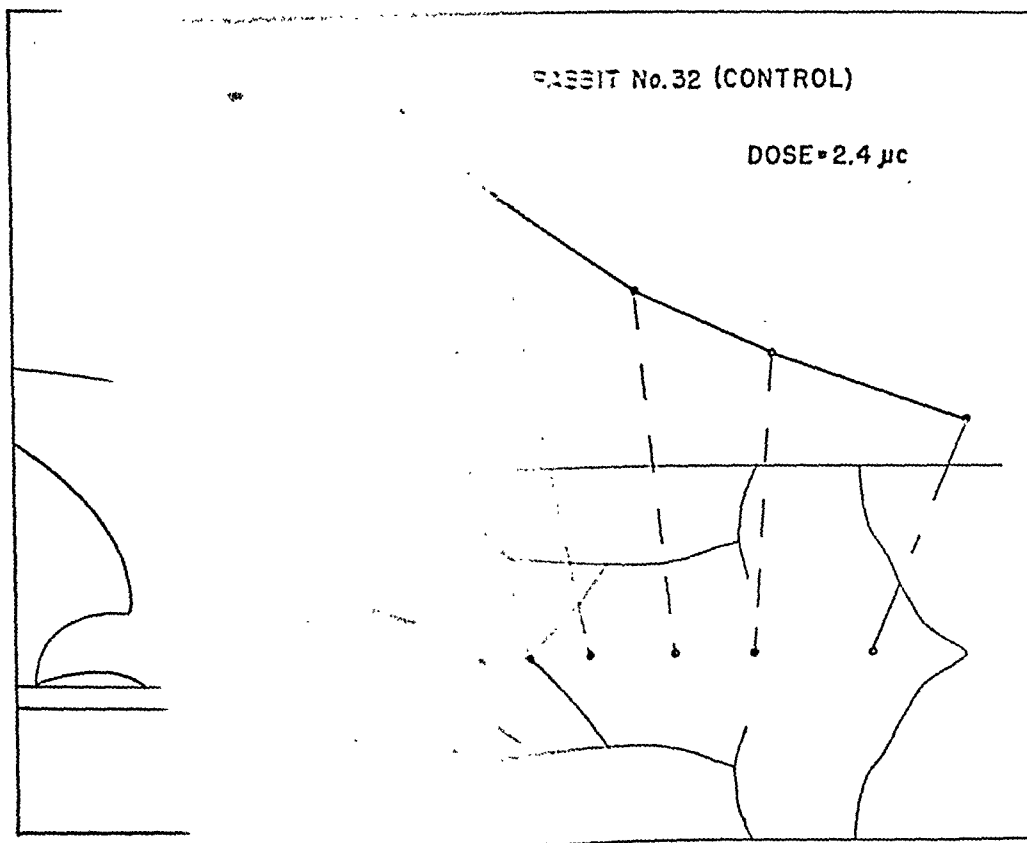


FIG. 2. CROSS-SECTIONS AS TO ADMINISTRATION. The position of

POINTS TAKEN FROM VARIOUS POINTS ON THE MIDLINE OF INJECTION OF RADIOACTIVE DI-BROM TRYPAN

from the xiphoid to the pelvis approxi-

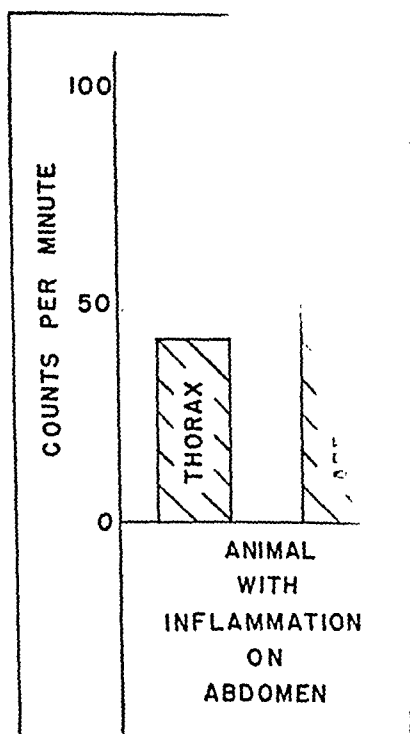


FIG. 3. CHART SHOWING THE CONTRAST BETWEEN THE THORAX AND ABDOMEN OF TWO ANIMALS WITH INFLAMMATION ON ABDOMEN CONTRAST TO THE CORRESPONDING RATES

This result can be produced with regularity. The average variation between normal sides, in a group of 6 normal animals in which leg lesions were taken, was 6.1 per cent. In an experimental group of 9 animals with leg lesions the increment produced by inflammation was significant. There were no failures.

The lesion is in the subcutaneous tissues of the abdominal wall, a different problem presents itself. The accumulation of radioactivity in the inflammatory process is superimposed on the high concentration of radioactivity in the abdomen. We cannot expect a significant increment in C.P.M. due to an inflammation when that process is small compared to the tissues which normally accumulate the injected radioactive material. The increase in C.P.M., due to an inflammation on the abdominal wall, may be 10 to 15 per cent. The point on the attention

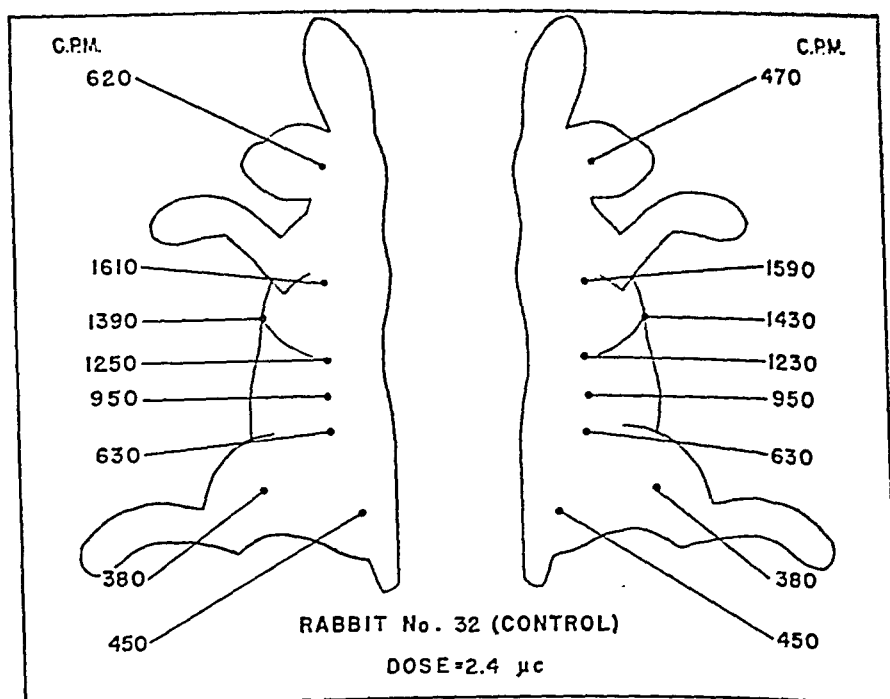


FIG. 4. DISTRIBUTION OF RADIOACTIVE DI-BROM TRYPAN BLUE IN A NORMAL RABBIT
Figures are in counts per minute, as registered from the various points shown.

whereas in the rat there is room for only two or three.

The distribution of radioactive di-brom trypan blue in the normal rabbit is shown in Figure 4. The bilateral symmetry of readings from various regions of the body should be noted. This symmetry is consistent within a variation of 10 to 20 per cent. Readings from the two sides of the head may vary to a greater degree than this because it is difficult to place the head of the anaesthetized animal in precisely the same position on the two sides. The same applies to the hind legs, where variations up to 25 per cent between normal sides may be observed.

If the counter is first centered over the upper thorax, in the midline, and then slowly moved down to the pelvis, taking readings each inch or so throughout its course, a set of readings are obtained which may be charted as shown in Figure 5. This indicates that the maximal concentration of dye is found in the region of the xiphoid under which are found the liver, spleen, heart, lungs, and superior splanchnic circulation, all closely grouped and accounting for much of the blood volume of the animal. Insofar as the

dye stays in the blood stream, largely, for the first 6 hours after injection, it is to be expected that this area will contain most of the dye. Furthermore, as the dye leaves the circulation, it is taken up by the reticulo-endothelial system (5), and as this same region contains the liver and spleen, it will continue to contain much of the dye even after the dye leaves the blood stream.

It is to be noted in Figure 5 that as the counter is moved down the abdomen of the animal, away from these viscera, the resultant chart is a straight line. That is, the decrease in C.P.M. is proportional to the distance from the xiphoid at which the count is taken.

These three points, then, bilateral symmetry, maximal counts over the xiphoid, and "linearity of decrease" of C.P.M. as the counter is moved toward the pelvis, are the chief normal findings of distribution of the dye in the animal from 1 to 6 hours after it is injected. The absolute values may vary; the pitch or absolute height of the abdominal "line" may vary, but symmetry of distribution, maximal counts over the xiphoid, and a straight line of decrease down the abdomen have been our findings in 53 observations on 15 control

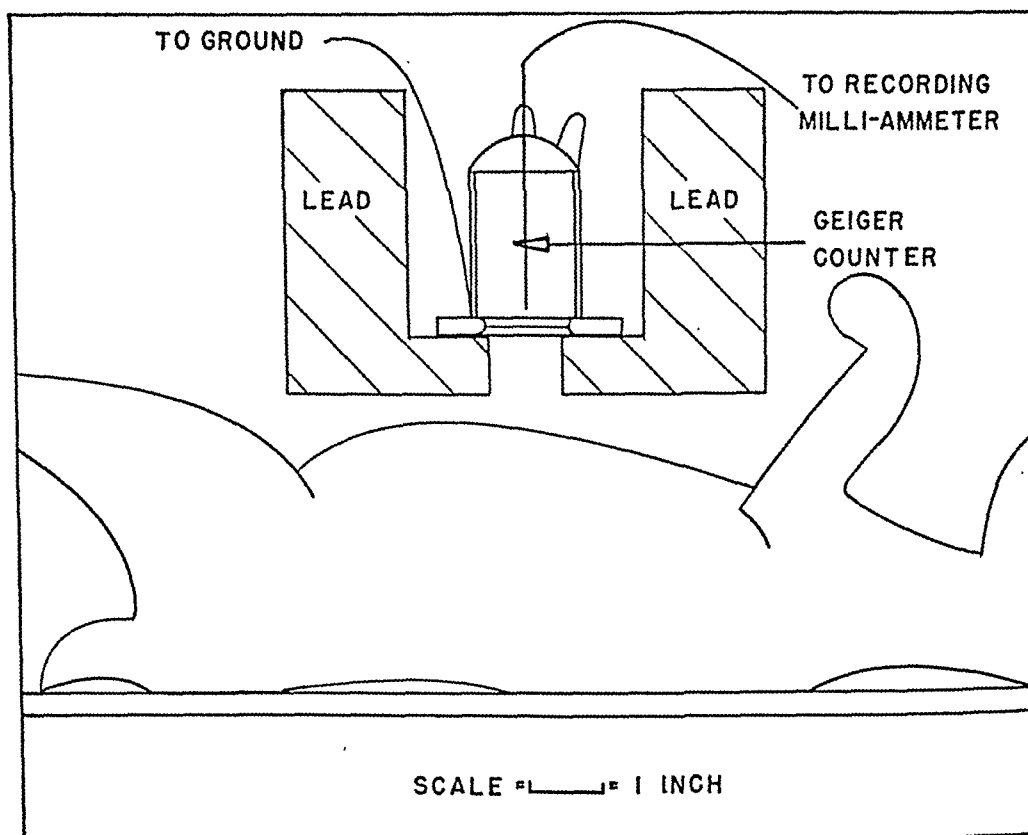


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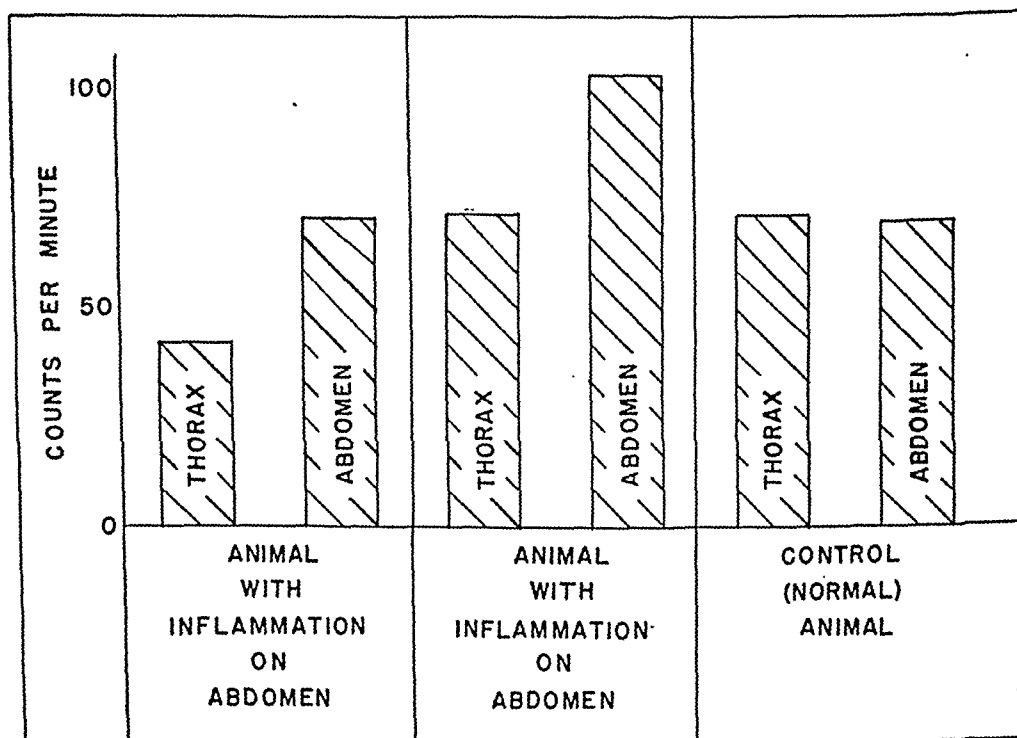


FIG. 3. CHART SHOWING THE NUMBER OF COUNTS PER MINUTE REGISTERED FROM THE THORAX AND ABDOMEN OF TWO RATS WITH INFLAMMATION ON THE ABDOMEN, IN CONTRAST TO THE CORRESPONDING READINGS FROM A NORMAL CONTROL ANIMAL.

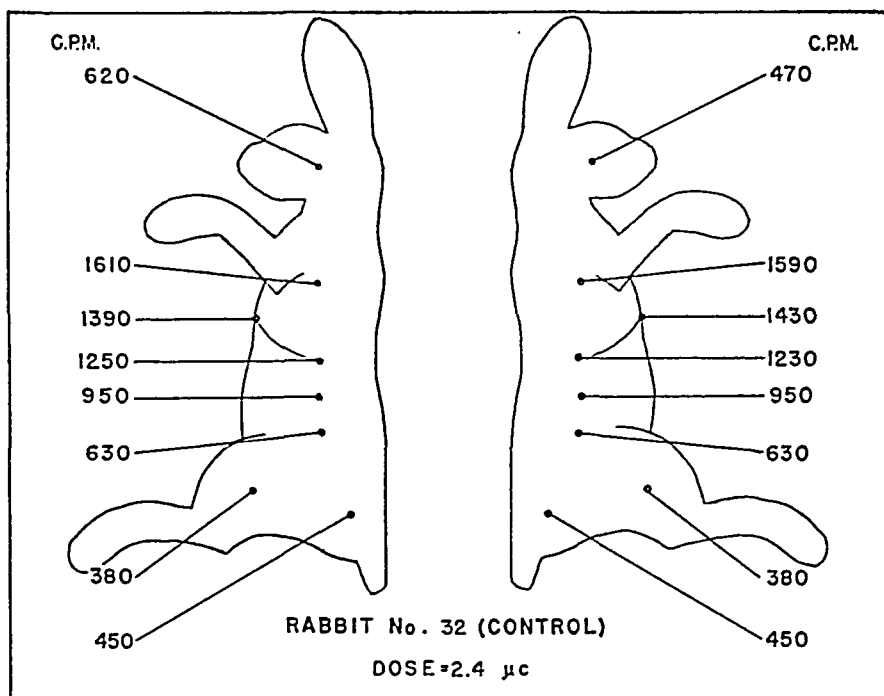


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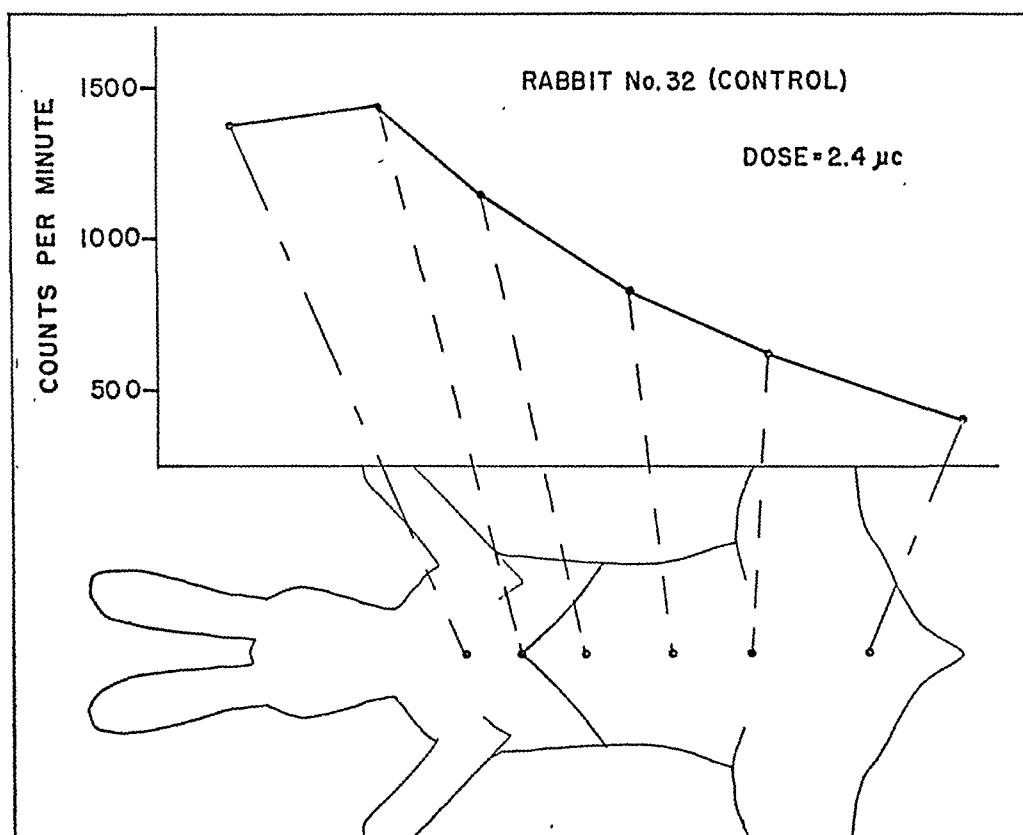


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The decline in readings as the counter is moved from the xiphoid to the pelvis approximates a straight line.

animals. In this same group, the average difference in C.P.M. between two normal sides was 10.9 per cent, the mean variation was 22.25 per cent, and the maximum (one instance) was 44.5 per cent.

3. Results in rabbits with inflammatory lesions

Our first study was devoted to lesions well away from the large blood-containing viscera in the upper abdomen. These lesions were on the hind legs where the accumulation of radioactivity due to the lesion would be superimposed on a normally low reading.

The findings in a rabbit with a hind-leg lesion are shown in Figure 6. The inflammatory lesion has produced an increment of 105 per cent more C.P.M. than registered from the contralateral normal leg.

This indicates concentration of the radioactive dye in the lesion to an extent detectable with the counter, and hence permitting diagnostic localization of the lesion by this technique.

This result can be produced with regularity. The average variation between normal sides, in a control group of 6 normal animals in which leg readings were taken, was 6.1 per cent. In an experimental group of 9 animals with leg lesions the average increment produced by inflammation was 91.0 per cent. There were no failures.

When the lesion is in the subcutaneous tissues of the abdominal wall, a different problem presents itself. The accumulation of radioactivity attendant upon the inflammatory process is superimposed on the high concentration of radioactivity normally found in the abdomen. We cannot expect a 90 per cent increment in C.P.M. due to an inflammatory process when that process is small and localized near tissues which normally accumulate a large proportion of the injected radioactive dye. In fact, the increase in C.P.M., due to an inflammatory process on the abdomen, may be only in the range of 10 to 15 per cent over the normal figure for that point on the animal.

However, if we turn our attention to the pattern

of distribution of the dye—the abdominal “line”—rather than the absolute C.P.M., we find that a significant distortion of that pattern may be produced by an inflammatory process.

It will be recalled that the normal linear decrease in C.P.M. is produced as the counter is moved caudad from the xiphoid (Figure 5). In the presence of an inflammatory process, this linear pattern may be distorted by having a “bump” in it, corresponding in location to the presence of the abscess. Such a distortion may be quite large and gross in character, as shown in Figure 7, or it may be a relatively small distortion, as shown in Figure 8. However, if the distortion of linearity constitutes an increase of 15 per cent or more over the expected figure (indicated by the dotted line), it is considered significant.

The data on inflammatory processes on the abdomen are based on 152 readings taken from 23 animals. In all these, “linearity” or its distortion was studied. In 7 normal animals, all showed normal charts of reading taken from the

abdomen. There were no departures from a straight line amounting to 10 per cent or more of the readings. The remaining 16 animals had inflammatory processes. Of these 10, or 62.5 per cent, showed positive distortion of the linear pattern. Six, or 37.5 per cent, showed essentially straight lines of decrease in C.P.M. as the counter was moved caudad from the xiphoid; in 2 cases, the lines were not straight but the distortion over the inflammation was inconsequential. Of the 6 animals in whom agar and staphylococci had been injected, but which did not accumulate dye to a detectable extent, 3 failed to show evidence of acute reaction to the organisms. There was no edema, heat, redness or evident vasodilatation. The remaining 3 negative animals showed sufficient inflammatory reaction to warrant expectation that they should have concentrated the dye. If we exclude the 3 animals which did not develop a local lesion, the percentage of positive results is 77 per cent for inflammatory processes under the skin of the abdomen.

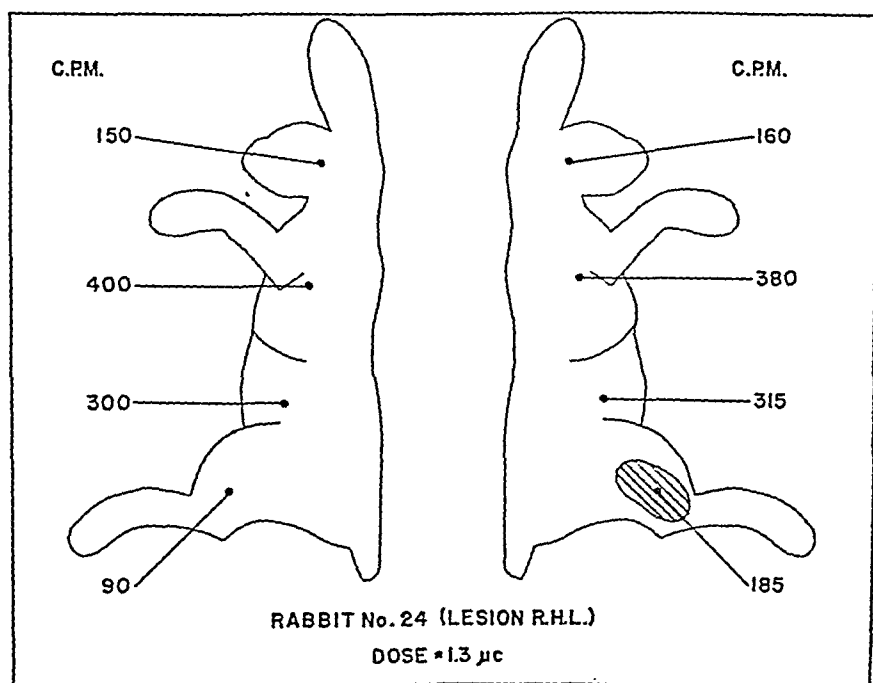


FIG. 6. FINDINGS IN A RABBIT WITH AN INFLAMMATORY PROCESS ON THE RIGHT HIND LEG

The animal has received an injection of $1.3\mu\text{c}$. of radioactive dye. The figures are in counts per minute from the various points shown. The inflammatory process has accumulated 105 per cent more dye than the control area on the other side.

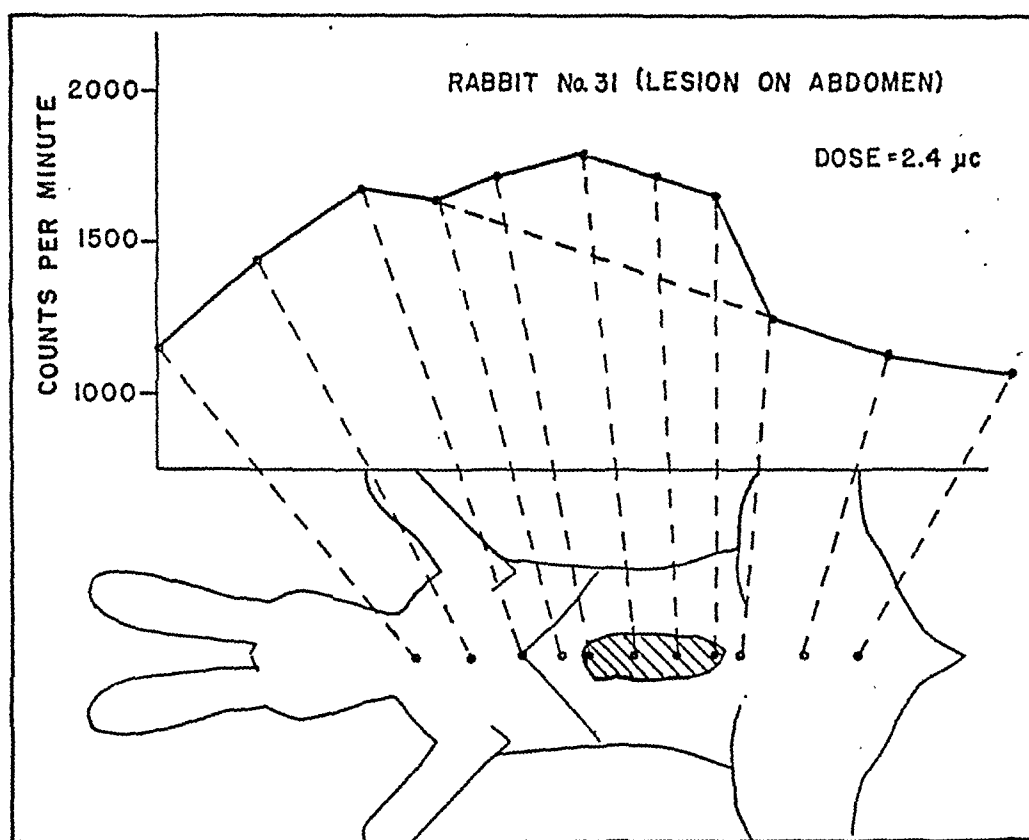


FIG. 7. CHART SHOWING THE READINGS TAKEN FROM VARIOUS POINTS ON THE MID-LINE OF A RABBIT WHICH HAS RECEIVED RADIOACTIVE DYE

There is an inflammatory process in the shaded area. The normally linear decline in readings is distorted by a rise over the inflamed area.

4. Experiments with bromide ion

In view of these findings with a dye made radioactive with radio-bromine, it is of interest to investigate the picture presented by the animal which has received an injection of radioactive bromine alone (as sodium bromide).

Weir and Hastings (21) have shown that bromide ion is distributed equally throughout the extracellular fluids. The readings from a normal animal which has received an injection of radio-bromide is shown in Figure 9. It will be noted that there is no significant decrease in readings as the counter moves caudad over the abdomen. The legs, furthermore, show somewhat higher readings than is usually found with radioactive dye. The abdominal readings are proportionately low for the magnitude of the leg readings, as compared with dye distribution.

The contrast of this picture with that of the distribution of the colloidal dye reflects the difference in distribution of a substance which is intravascular, from one which is partitioned throughout all the extracellular fluid. The intra-

vascular substance shows apparent concentration around the large blood-containing viscera, whereas the ion distributed throughout extracellular fluid shows a distribution more nearly proportional to the simple cross-section of the body at that point. This corroborates the conclusion drawn from the chemistry of the dye, that the "tracer" is being carried through the body by the larger organic molecule of which it is a part, rather than assuming a distribution of its own.

Our results indicate that bromide ion concentrates to a slight extent in inflammatory processes, probably in the edema fluid.

5. Measurements of tissue radioactivity

Another approach to this problem is the excision of tissue from the inflamed area, and the study of the amount of radioactivity it has taken up, in contrast to that contained in adjacent normal tissue. To do this, the excised tissue is reduced to an ash in the presence of silver and its radioactivity, as silver bromide, is read directly under the counter. In rabbit No. 31, which gave un-

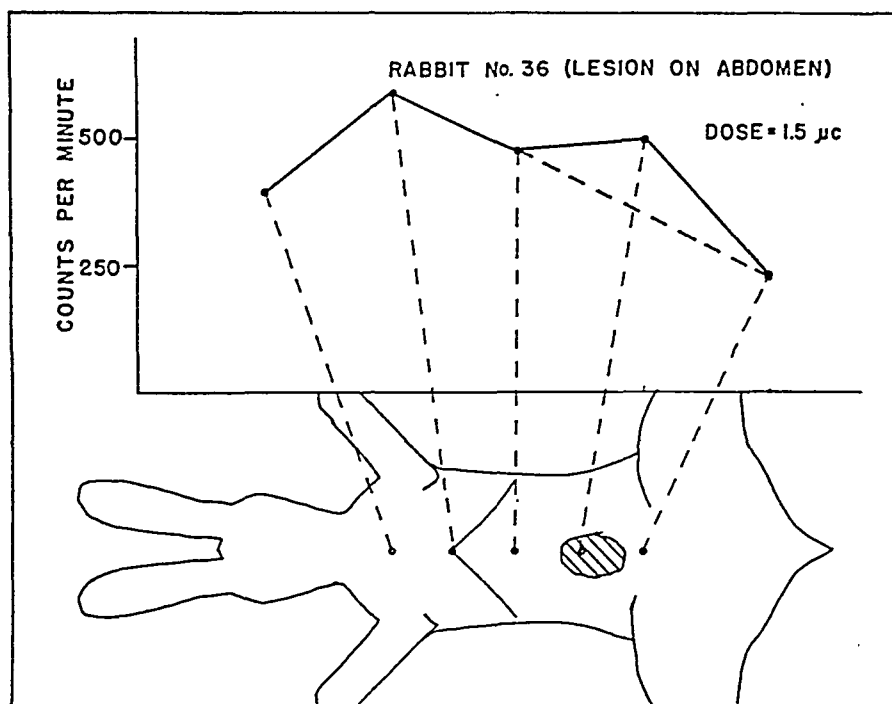


FIG. 8. CHART SHOWING THE READINGS TAKEN FROM VARIOUS POINTS ON THE MID-LINE OF A RABBIT WHICH HAS RECEIVED RADIOACTIVE DYE

There is an inflammatory process in the shaded area. The normally linear decline in readings has been distorted by the inflammation but to a lesser extent than in the animal shown in Figure 7.

mistakable evidence of the presence of its abscess by the accumulation of radioactive dye (Figure 7), approximately 110 per cent more radioactivity per gram of tissue was found in the area surrounding the inflammatory process than in the neighboring normal tissue. In another animal, in which the original counter readings gave little evidence as to the whereabouts of the abscess, dissection revealed a very quiescent process, and only 40 per cent more radioactive dye per gram of tissue was found in the tissues around the agar nidus, than in adjacent normal tissue. Evidently the animal's resistance to the organism was such as to preclude the appearance of an abscess at the site of injection, and thus to prevent the accumulation there of enough dye to increase the counter reading.

6. Discussion

Radioactive di-brom trypan blue gives us a means of diagnosing the location of abscesses on the legs or in the subcutaneous tissues of the

abdominal wall of the rabbit. The leg lesions present a fairly simple problem, and so long as there is any inflammation there, enough dye will accumulate to make the lesion evident on the counter. The abdominal lesions, on the other hand, present a less favorable situation, and the results are less gratifying, since in only 77 per cent of the animals could a positive correlation be made of the presence of a lesion and localization of a detectable amount of dye. In the case of the abdominal lesions, the inflammatory process must be quite intense in order to accumulate enough dye to be demonstrable from outside the body.

The failure of some of the animals to concentrate a detectable amount of dye in these lesions is attributable to three factors:

(1) There is a normal variation in the ability of animals to concentrate the dye in inflammatory lesions, as noted by previous workers with dye (9a).

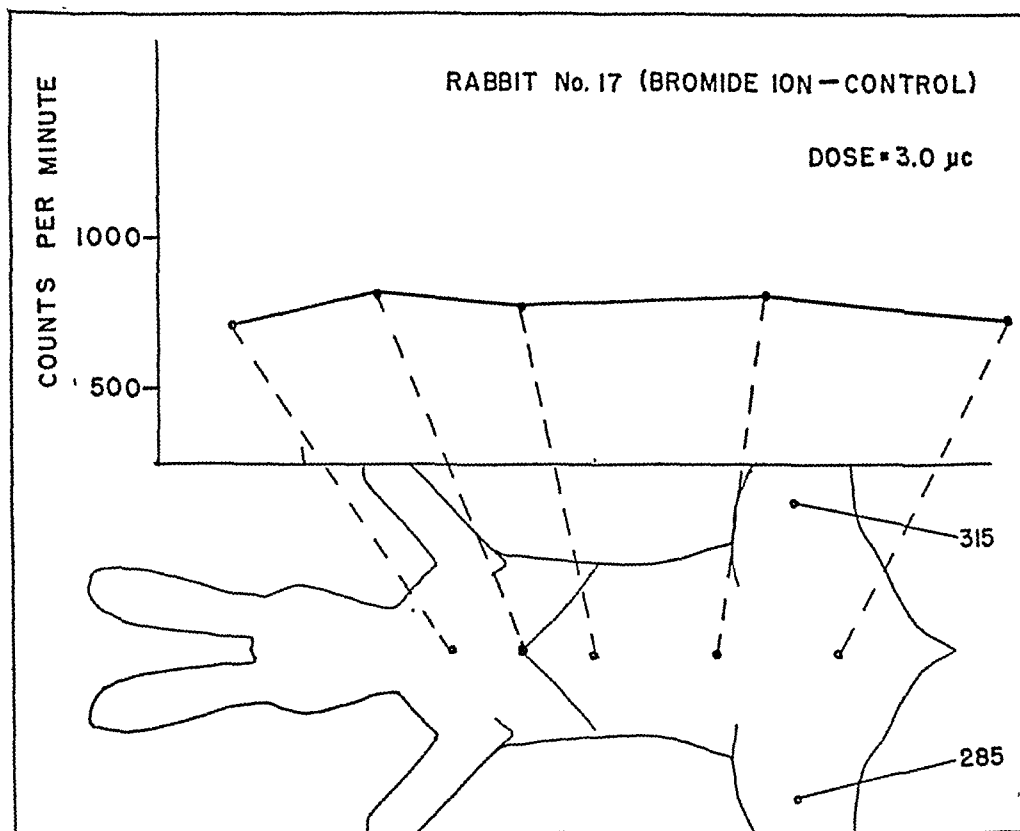


FIG. 9. CHART SHOWING THE READINGS TAKEN FROM VARIOUS POINTS ON THE MID-LINE AND LEGS OF A RABBIT WHICH HAS RECEIVED AN INJECTION OF RADIOACTIVE BROMINE AS SODIUM BROMIDE

There is only a slight decrease in readings as the counter moves down the abdomen. The leg readings are relatively higher than those found using radioactive dye.

(2) Variations in virulence of the organism or resistance of the animal may lead to such a mild process that the dye is not accumulated.

(3) This particular dye does not seem to concentrate as consistently in inflammatory lesions as does its non-brominated counterpart, trypan blue.

The first two of these factors are important whenever one attempts to simulate human lesions with staphylococcal abscesses. Virulence and resistance cannot be closely enough controlled to produce unfailing abscess-formation. Properties of the dye may also be an important factor in its failure to accumulate in lesions on some of the animals. As previously pointed out, the molecular weight has been increased, the dye is less soluble in water, and some particulate dye may be filtered out in lungs or liver and thus lost to the inflammatory area. Shortcomings such as these, inherent in the dye itself, can only be overcome by further study of this dye or of other similar dyes such as

its isomer, radioactive di-brom Evans blue. Such study is in progress at present.

SUMMARY

(1) A study of the distribution of radioactive di-brom trypan blue in normal and inflamed animals is described.

(2) This radioactive colloidal dye concentrates in inflammatory lesions to an extent detectable from outside the intact animal with a suitable counter.

(3) Using this radioactive dye, lesions in the periphery of the body were detectable in all cases, whereas abdominal lesions were detectable in 77 per cent of instances.

The authors wish to express their gratitude to Dr. Joseph C. Aub, Dr. Waldo E. Cohn and Dr. Austin M. Brues for their continued interest and valuable advice, and to Prof. Louis F. Fieser for his assistance in regard to the chemistry involved.

BIBLIOGRAPHY

1. Goldmann, E. E., Die Äussere und Innere Sekretion des Gesunden und Kranken Organismus im Lichte der "vitale Färbung." Beitr. z. Klin. Chir., 1909, 64, 192. Quoted by Burrows (11).
2. Bowman, F. B., Winternitz, M. C., and Evans, H. M., Ueber die vitale Färbung des Tuberkels. Centralb. f. Bakt., 1912, 65, 403.
3. Kline, R. S., and Winternitz, M. C., Studies upon experimental pneumonia in rabbits. VIII. Intravital staining in experimental pneumonia and the circulation in the pneumonic lung. J. Exper. Med., 1915, 21, 311.
4. MacCurdy, J. T., and Evans, H. M., Experimentelle Läsionen des Centralnervensystems, untersucht mit Hilfe der vitalen Färbung. Berl. Klin. Wchnschr., 1912, 49, 1695.
5. Evans, H. M., The macrophages of mammals. Am. J. Physiol., 1915, 37, 243.
6. Evans, H. M., and Schulemann, W., The action of vital stains belonging to the benzidine group. Science, 1914, 39, 443.
7. Duran-Reynals, F., A general permeability-increasing effect of a factor from mammalian testicle on blood capillaries. Yale J. Biol. and Med., 1939, 11, 601.
8. Menkin, V., Studies on inflammation. I. Fixation of vital dyes in inflamed areas. J. Exper. Med., 1929, 50, 171.
9. Menkin, V., The Dynamics of Inflammation. Macmillan, New York, 1940.
- 9a. Menkin, V., Personal communication.
10. Rigdon, R. H., Capillary permeability in the skin of the rabbit. Proc. Soc. Exper. Biol. and Med., 1939, 42, 43.
11. Burrows, H., Some Factors in the Localization of Disease in the Body. Wm. Wood and Co., New York, 1932.
12. Keith, N. M., Rowntree, L. G., and Geraghty, J. T., A method for the determination of plasma and blood volume. Arch. Int. Med., 1915, 16, 547.
13. Gibson, J. G., Jr., Personal communication.
14. Dawson, A. B., Evans, H. M., and Whipple, G. H., Blood volume studies. III. The behaviour of a large series of dyes introduced into the circulating blood. Am. J. Physiol., 1920, 51, 232.
15. Gregerson, M. I., Gibson, J. G., and Stead, E. A., Plasma volume determinations with dyes; errors in colorimetry; use of the blue dye T-1824. Am. J. Physiol., 1935, 113, 54.
16. Strauss, S. F., Neuwelt, F., Rovner, L., and Necheles, H., A new method for the detection of hidden abscesses. Surgery, 1938, 4, 930.
17. Kroll, H. H., Strauss, S. F., and Necheles, H., Concentration and detection of a dye in abscesses. Proc. Soc. Exper. Biol. and Med., 1940, 43, 228.
18. Kroll, H. H., Strauss, S. F., and Necheles, H., Studies on the detection of abscesses and tumors. III. Concentration and detection of a radioactive substance in abscesses. J. Lab. and Clin. Med., 1941, 27, 50.
19. Seaborg, G. T., Artificial radioactivity. Chem. Rev., 1940, 27, 199.
20. Tobin, L. H., and Moore, F. D., Studies with radioactive di-azo dyes. III. The synthesis and properties of radioactive di-brom trypan blue and radioactive di-brom Evans blue. In preparation.
21. Weir, E. G., and Hastings, A. B., The distribution of bromide and chloride in tissues and body fluids. J. Biol. Chem., 1939, 129, 547.

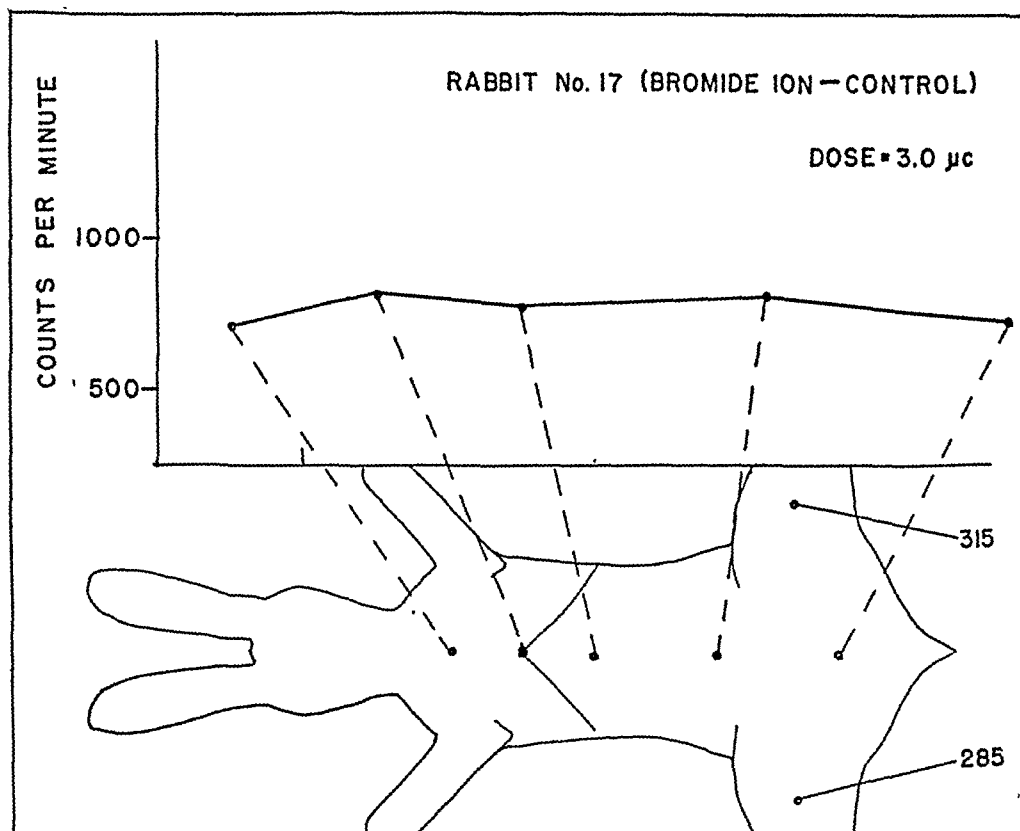


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There is only a slight decrease in readings as the counter moves down the abdomen. The leg readings are relatively higher than those found using radioactive dye.

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BIBLIOGRAPHY

1. Goldmann, E. E., Die Äussere und Innere Sekretion des Gesunden und Kranken Organismus im Lichte der "vitale Färbung." Beitr. z. Klin. Chir., 1909, 64, 192. Quoted by Burrows (11).
2. Bowman, F. B., Winternitz, M. C., and Evans, H. M., Ueber die vitale Färbung des Tuberkels. Centralb. f. Bakt., 1912, 65, 403.
3. Kline, R. S., and Winternitz, M. C., Studies upon experimental pneumonia in rabbits. VIII. Intra-vitam staining in experimental pneumonia and the circulation in the pneumonic lung. J. Exper. Med., 1915, 21, 311.
4. MacCurdy, J. T., and Evans, H. M., Experimentelle Läsionen des Centralnervensystems, untersucht mit Hilfe der vitalen Färbung. Berl. Klin. Wchnschr., 1912, 49, 1695.
5. Evans, H. M., The macrophages of mammals. Am. J. Physiol., 1915, 37, 243.
6. Evans, H. M., and Schulemann, W., The action of vital stains belonging to the benzidine group. Science, 1914, 39, 443.
7. Duran-Reynals, F., A general permeability-increasing effect of a factor from mammalian testicle on blood capillaries. Yale J. Biol. and Med., 1939, 11, 601.
8. Menkin, V., Studies on inflammation. I. Fixation of vital dyes in inflamed areas. J. Exper. Med., 1929, 50, 171.
9. Menkin, V., The Dynamics of Inflammation. Macmillan, New York, 1940.
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10. Rigdon, R. H., Capillary permeability in the skin of the rabbit. Proc. Soc. Exper. Biol. and Med., 1939, 42, 43.
11. Burrows, H., Some Factors in the Localization of Disease in the Body. Wm. Wood and Co., New York, 1932.
12. Keith, N. M., Rowntree, L. G., and Geraghty, J. T., A method for the determination of plasma and blood volume. Arch. Int. Med., 1915, 16, 547.
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15. Gregerson, M. I., Gibson, J. G., and Stead, E. A., Plasma volume determinations with dyes; errors in colorimetry; use of the blue dye T-1824. Am. J. Physiol., 1935, 113, 54.
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18. Kroll, H. H., Strauss, S. F., and Necheles, H., Studies on the detection of abscesses and tumors. III. Concentration and detection of a radioactive substance in abscesses. J. Lab. and Clin. Med., 1941, 27, 50.
19. Seaborg, G. T., Artificial radioactivity. Chem. Rev., 1940, 27, 199.
20. Tobin, L. H., and Moore, F. D., Studies with radioactive di-azo dyes. III. The synthesis and properties of radioactive di-brom trypan blue and radioactive di-brom Evans blue. In preparation.
21. Weir, E. G., and Hastings, A. B., The distribution of bromide and chloride in tissues and body fluids. J. Biol. Chem., 1939, 129, 547.

THE SERUM ANTISTREPTOLYSIN TITER IN CHRONIC GLOMERULONEPHRITIS

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Previous reports (1, 2, 3) have dealt with the serum antistreptolysin titer response in acute glomerulonephritis. Seegal, Lyttle and their associates (2) found that 94 per cent of 116 consecutive cases of acute glomerulonephritis were associated with significant rises in antistreptolysin titer. Longcope (3) found an increased antistreptolysin titer in the acute stage of 72 per cent of 36 cases of acute hemorrhagic nephritis (type A). These studies show that the *acute* infections, which so typically precede the onset of acute glomerulonephritis, are generally due to the group A hemolytic streptococcus. Winkenwerder, McLeod and Baker (4), however, described another type of glomerulonephritis (type B), characterized by an insidious onset of edema and a progressive downhill course, and usually associated with *chronic* upper respiratory infection. Although group A hemolytic streptococci were often recovered from the upper respiratory passages of these patients (type B), Longcope (3) showed that rises in antistreptolysin titer were a rarity. Indeed, the titers were frequently found to be abnormally low.

The present study is based on the examination of the serum antistreptolysin titer response in patients with chronic glomerulonephritis. The case material consists of 81 patients who have been studied at the Nephritis-Hypertension Clinic of Dr. Dana W. Atchley and Dr. Robert F. Loeb at the Presbyterian Hospital, a similar clinic of Dr. John D. Lyttle at the Babies Hospital, and at the Research Service of the First Division of the Welfare Hospital.

This study was undertaken with full knowledge of the difficulty of establishing the diagnosis of chronic glomerulonephritis. In this series of 81 patients presenting the picture of chronic glomerulonephritis, 24 are known to have died. Autopsies performed on 13 of these revealed chronic glo-

merulonephritis. A biopsy of the kidney established the diagnosis in one additional case. In 23 other cases, attacks typical either of acute glomerulonephritis, *i.e.*, the onset of the disease, or of an exacerbation of chronic glomerulonephritis, have been observed. Thus, the diagnosis of glomerulonephritis seems to have been established beyond doubt in 37 of the 81 cases. The remaining 44 patients were thoroughly studied and, in the opinion of several experienced observers, there was no evidence of renal disease other than chronic glomerulonephritis.

The patients comprising this study have been observed over periods of from 4 months to 8 years. Twelve patients were followed for less than one year, the short follow-up being due to early death in 10 instances. Fifty-two, or 64 per cent, were followed for 2 or more years. Sera for the determination of the antistreptolysin titer were obtained at varying intervals in each patient. In hospitalized cases, determinations were made once or twice a week. It was not possible to obtain tests with this frequency in the ambulatory patients seen at the clinic. However, in the majority of these instances, determinations were made at intervals of from 1 to 4 months. In addition, the patients were instructed to report to the clinic whenever an infection developed and were then studied weekly.

Todd (5) developed the technique for the determination of serum antistreptolysin and with minor modifications (2) this technique has been employed throughout the present studies. The antistreptolysin value is determined by the minimum amount of serum necessary to neutralize a standard amount of streptolysin. The value may be recorded either as the amount of serum in fractions of cubic centimeters necessary for the neutralization of the streptolysin, or as units which are calculated as the reciprocal of the frac-

tion of cubic centimeters. The latter method was used throughout this study. An increase in serum antistreptolysin value may be represented as the maximum value attained in the rise or as the rise in titer over base-line determinations in individual patients.

A significant increase in serum antistreptolysin has been shown to indicate group A hemolytic streptococcal infection, since a variety of infections and other diseases are not followed by increases in the titer of this antibody (3, 5, 6). Mote and Jones (6), in a study of the antistreptolysin titer response of 811 "healthy" subjects and 525 "sick" individuals, state that, "... in no instance was the titer observed to increase in an individual in whom infection by the hemolytic streptococcus could be definitely excluded."

CRITERIA FOR RISE IN ANTISTREPTOLYSIN TITER

In order that a rise in serum antistreptolysin titer be considered significant, it was required that:

1. The increase in the titer reading be represented by at least 2 decrements of 0.1 ml. of standard diluted serum over well-established base-line determinations for the individual patient. With the exception of 1 instance, each rise in the present study accepted as significant was based on 3 or more such decrements.

2. The individual titer values of the rise oc-

cur in a progressively increasing or decreasing (or both) curve. Thus, erratic fluctuations in titer (as shown in Figure 1) were not accepted as significant.

In order to determine the true maximum value of a rise in antistreptolysin titer, it was further required that at least one test be available within 3 weeks of the onset of an infection; or, in a few instances, that the antistreptolysin titer curve be still rising toward a maximum, if the first titer of the rise was determined on serum withdrawn more than 3 weeks after the onset of an infection.

SUMMARY OF MATERIAL

There were 101 significant antistreptolysin titer rises occurring in 61 cases. Eighty-five of these rises form the basis of this study.¹

¹ In addition to the 16 rises in titer whose maxima were not apparent, 36 other instances of variations in antistreptolysin titer were discarded for the following reasons: (a) In two instances, clear cut end points in the antistreptolysin titrations could not be obtained. In both cases, the sera were very lipemic. In one, the rise was associated with a group A hemolytic streptococcus tonsillitis, while no infection was present in the other. (b) In 17 instances, the variations in antistreptolysin titer fluctuated without a definite trend being apparent. Several such fluctuations may be seen in Figure 1. Eight of these occurred in patients with edema. In 11 instances, there was no evidence of infection, while the remaining 6 gave histories of frequent head colds. In no instances

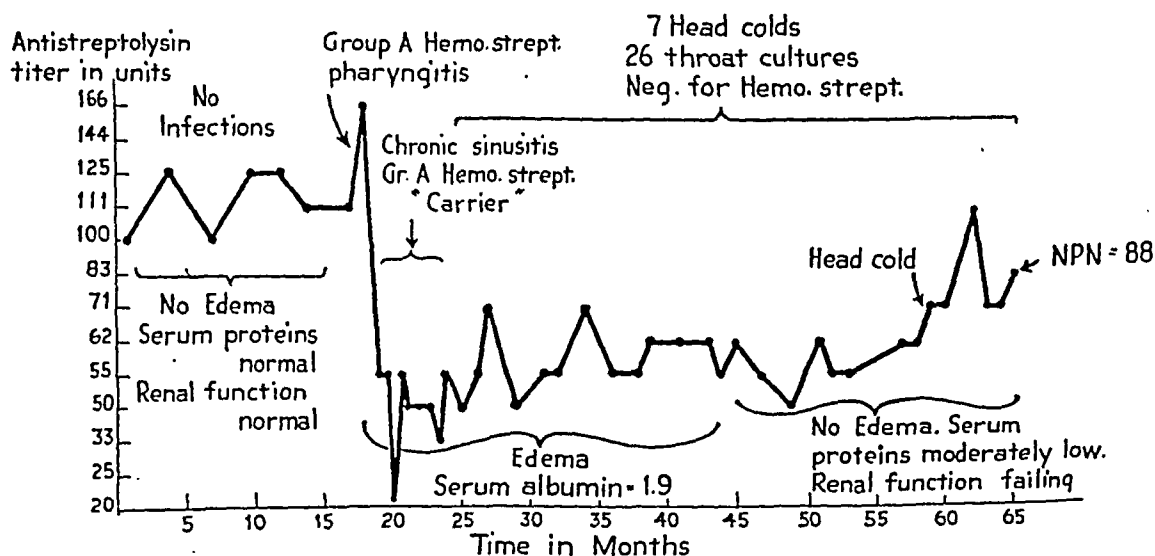


FIG. 1. ANTISTREPTOLYSIN TITER CURVE OF PATIENT WITH CHRONIC GLOMERULONEPHRITIS SHOWING LOW TITERS ASSOCIATED WITH ONSET OF NEPHROTIC STAGE, ALTHOUGH GROUP A HEMOLYTIC STREPTOCOCCI WERE PRESENT IN THROAT CULTURES DURING FIRST 6 MONTHS OF NEPHROTIC STAGE

It should be noted that there were no variations of antistreptolysin titer in 8 of the 81 patients with chronic glomerulonephritis. These 8 patients were followed over periods of from 8 to 102 months, with an average of 35 months per patient. An average of 21 antistreptolysin determinations per patient was made, with a range of 7 to 84. An analysis of these cases indicated no striking differences in their clinical course as compared with that of the cases showing rises in titer.

The data presented in the foregoing sections are summarized in Table I.

TABLE I

Summary of antistreptolysin titer data in 81 cases of chronic glomerulonephritis

- 101 definite rises in 61 cases.
- 85 rises in 56 cases, with sufficient data to indicate the maximum titer of the rise.
- 16 rises with insufficient data to indicate the maximum titer of the rise.
- 36 questionable rises in 28 cases (includes some of the cases above).
- 8 cases with no variation of antistreptolysin titer in period of study (8 to 102 months).

RELATION OF SEX AND AGE TO ANTISTREPTOLYSIN TITER RESPONSE

Sex did not appear to modify the antistreptolysin titer response. Of the 81 cases analyzed, 49 were males and 32 females. Forty-eight of the adequately studied rises in antistreptolysin titer occurred in male patients, 37 in female patients. Table II summarizes the sex incidence in relation to the maximum values of the antistreptolysin titer rises.

With respect to age, the rises in antistreptolysin titer were, in general, greater among the children than among the adults. These data are sum-

TABLE II

Sex distribution of small, medium and large rises in antistreptolysin titer in chronic glomerulonephritis

Maximum titer of rise	Number in 35 male patients	Number in 21 female patients
125 units or less (small).....	24	16
144 units to 250 (medium).....	14	15
333 units or more (large).....	10	6

were the throat cultures positive for hemolytic streptococci. (c) In 17 instances, the rise in titer was indicated by a single determination. In 12 of these, there were gaps of 2 or more months between the titer showing the rise and the nearest base-line determinations.

TABLE III

Age distribution of small, medium and large rises in antistreptolysin titer in chronic glomerulonephritis

Maximum titer of rise	Number of rises in 5 patients, 1 to 9 years of age	Number of rises in 28 patients, 10 to 19 years of age	Number of rises in 48 patients, 20 or more years of age
125 units or less.....	0	17	23
144 units to 250.....	7	7	15
333 units or more....	4	9	3
Total rises.....	11	33	41

marized in Table III. It is possible that the severity of the hemolytic streptococcal infections may have played a role in this difference, but the number of children in this series was not large enough to determine this point.

RELATION OF INFECTION TO ANTISTREPTOLYSIN TITER RESPONSE

A culture was taken of each patient's throat at every visit to the clinic. Routine throat cultures were taken of hospitalized patients twice a week. Cultures were taken daily or every other day in hospitalized patients if there was any suspicion of infection. In addition, the patient was questioned in regard to the symptoms indicative of infection, and examinations of the nose, pharynx and cervical lymph nodes were made at each clinic visit, and at appropriate intervals in hospitalized cases. The throat cultures were classified as positive or negative for group A hemolytic streptococci. Infections, if present, were classified as chronic or acute. The acute infections were further subdivided into "deep" or "superficial" groups, following the scheme previously reported (1). Deep infections included mastoiditis, peritonsillar or cervical abscess, moderate or severe cervical lymphadenitis, otitis media and sinusitis. Superficial infections included pharyngitis (usually with mild cervical adenitis) and the "common cold."

Fifteen of the 85 adequately studied rises in antistreptolysin titer were associated with deep, 67 with superficial, and 3 with no observed infections. Twenty per cent of the rises in titer following deep infections attained a maximum of 333 units or more (large rises), 33 per cent reached from 144 to 250 units (medium rises), and 47 per cent reached a maximum of 125 units or less (small rises). The superficial infections

showed an almost identical distribution, namely 19, 33 and 48 per cent, followed respectively by large, medium and small rises in antistreptolysin titer. In summary, the magnitude of the antistreptolysin titer response among these patients with chronic glomerulonephritis was unrelated to the deep or superficial character of hemolytic streptococcal infection.

The percentage of rises in antistreptolysin titer associated with the presence of group A hemolytic streptococci and the presence or absence of infection are summarized in Table IV. Since a rise

TABLE IV

Percentage of small, medium and large antistreptolysin titer rises associated with cultures showing Group A hemolytic streptococci and with infections in patients with chronic glomerulonephritis

Maximum titer of rise	Number of rises	Rises with Group A hemolytic streptococci in cultures	Rises with infection		
			Acute	Chronic	None observed
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
125 units or less...	40	42	78	20	2
144 units to 250...	29	52	79	14	7
333 units or more...	16	75	100	0	0
All rises.....	85	52			

in antistreptolysin titer is confined to infections caused by hemolytic streptococci, usually Group A, it is apparent that this test was of definite value in determining the etiology of many infections occurring in patients with chronic glomerulonephritis.

These data indicate that rises in antistreptolysin titer that attain a maximum of 333 units or more are associated with a higher incidence of cultures showing group A hemolytic streptococci than are the smaller rises. Likewise, the greater rises are all associated with acute infection, while 22 per cent of the small rises and 21 per cent of the medium rises are associated either with chronic infection or with no observed infection.

GROUP A HEMOLYTIC STREPTOCOCCAL INFECTION WITHOUT RISE IN ANTISTREPTOLYSIN TITER

In this series of 81 patients with chronic glomerulonephritis, there were 5 acute infections (1, otitis media; 1, bronchitis; and 3, pharyngitis) apparently due to group A hemolytic streptococci,

wherein adequate antistreptolysin titer studies revealed no subsequent rise in titer. Three of these occurred in patients with edema, 2 in patients without edema. All but one of these infections occurred in patients who exhibited rises in titer at other times. Two of these infections occurred at a time when the titer was declining from a previous rise.

Several other acute group A hemolytic streptococcal infections were observed in which adequate antistreptolysin titer data were not obtained. On this account, these infections could not be included in the analysis of the antibody response.

GROUP A HEMOLYTIC STREPTOCOCCUS "CARRIER STATE"

There were 26 instances of the "carrier state" occurring in 20 patients. The "carrier state" in this study refers, arbitrarily, to instances in which group A hemolytic streptococci were recovered from the throats of individuals without evidence of *acute* upper respiratory infection and without significant rise in antistreptolysin titer. Occasionally only 1 or 2 isolated throat cultures were positive for hemolytic streptococci, but usually repeated cultures over periods of several weeks to as long as 6 months were positive. These "carrier states" were frequently but not invariably associated with *chronic* sinusitis and post-nasal discharge. It is recognized that these "carrier states" may represent actual hemolytic streptococcal infection. However, this seems unlikely in the majority of instances inasmuch as 18 of these 20 patients had responded to hemolytic streptococcal infections at other times with definite rises in antistreptolysin titer.

EFFECT OF NEPHROTIC PHASE ON ANTISTREPTOLYSIN TITER RESPONSE

Edema associated with low serum albumin and high serum cholesterol concentrations was considered to indicate the nephrotic phase of chronic glomerulonephritis. There were marked variations in the degree of edema and in the levels of serum albumin and cholesterol among the different cases, as well as in particular individuals. The problem was further confused by the occurrence, in some cases, of edema due to circulatory failure. In a few instances there was a possibility that

the edema and hypoproteinemia were due to malnutrition. However, the status of each patient at the time of a significant rise in antistreptolysin titer was evaluated and classified, to the best of our ability, as being either non-nephrotic or nephrotic. When this was done, it became apparent that the antistreptolysin titer responses to hemolytic streptococcal infections were significantly greater among the patients without edema than among those with edema. This is illustrated by Figure 2.

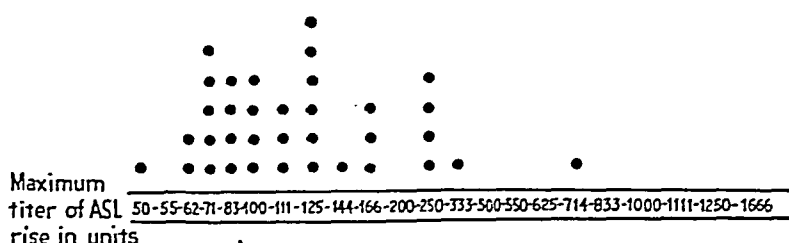
In addition, it should be noted that 18 of 26 group A hemolytic streptococcal carrier states oc-

curred in patients with the nephrotic element. Three of the 5 proven group A hemolytic streptococcal infections, not followed by a rise in titer, were also in the nephrotic group.

Although, as was previously indicated, there was a higher incidence of the greater rises in antistreptolysin titer among the younger patients than among the older groups, the height of the rise depended on the presence or absence of edema, as well as on the age distribution. This is illustrated in Table V.

Many patients with chronic glomerulonephritis have been observed during periods of transition

35 Rises in Patients with the Nephrotic Stage



50 Rises in Patients without the Nephrotic Stage

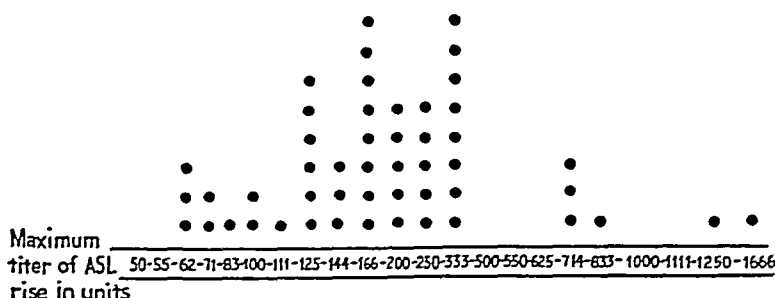


FIG. 2. DISTRIBUTION OF MAXIMA OF ANTISTREPTOLYSIN TITER RISES IN PATIENTS WITH CHRONIC GLOMERULONEPHRITIS, WITH AND WITHOUT EDEMA

TABLE V

Age distribution of small, medium and large rises in antistreptolysin titer occurring among patients with chronic glomerulonephritis and edema

Maximum titer of rise	Patients 9 years of age and under		Patients 10 to 19 years of age		Patients 20 and more years of age	
	Number of rises in cases with edema	Number of rises in cases without edema	Number of rises in cases with edema	Number of rises in cases without edema	Number of rises in cases with edema	Number of rises in cases without edema
125 units or less.....	0	0	12	5	13	10
144 units to 250.....	2	5	1	6	5	10
333 units or more.....	1	3	1	8	0	3

from the dry to the edematous phase of the disease, or *vice versa*. In 9 of these instances studied, significant changes in the base line level of the antistreptolysin titer were noted, the titer being higher in the non-edematous than in the edematous phase. Figure 1 is an illustration of this point. However, in other instances, similar changes in base line titers were not associated with changes from the nephrotic to non-nephrotic phase of chronic glomerulonephritis, or *vice versa*.

DISCUSSION

A rise in serum antistreptolysin titer was found to be of definite value in detecting group A hemolytic streptococcal infections, in the presence of negative throat cultures, among patients with chronic glomerulonephritis, as well as being of confirmatory value when the cultures were positive. This test was not infallible, since there were 5 acute infections associated with positive cultures for group A hemolytic streptococci which were not associated with rises in titer.

Males outnumbered females in this series. Sex did not significantly modify the antistreptolysin titer response. No previous study on antistreptolysin titer in various diseases has noted any positive effect of sex on the test.

With respect to age, the younger patients had, in general, greater rises in antistreptolysin titer following infection than did the older patients. Lyttle and his colleagues (2) reported a similar correlation in their study of the serum antistreptolysin titer in acute glomerulonephritis. Lippard and Johnson (7), however, found that rises in antistreptolysin titer following hemolytic streptococcal infections among children more than 17 months of age were comparable to those observed in adults. Infants under 17 months of age, in contrast, showed little tendency to exhibit rises in antistreptolysin titer. In cases of chorea, Bunim and McEwen (8) found "no strict correlation between the age of the patient, the severity of the chorea, and the height of the antistreptolysin titer." Mote and Jones (6) found that the incidence of rises in antistreptolysin titer was greater in younger "healthy" control subjects than in the older age groups, presumably because of "a higher incidence of very mild or subclinical respiratory infections in children." In their rheumatic

patients, age had no effect on the incidence of antistreptolysin titer response. They also stated that age had no effect on the magnitude of the response in these patients.

In the present series of 81 patients with chronic glomerulonephritis, it was found that the greater the magnitude of the rise in antistreptolysin titer, the greater was the frequency of positive cultures for group A hemolytic streptococci. It was also noted that the incidence of preceding acute infection was higher in the group showing larger rises in titer, than among those exhibiting the medium and small rises. However, whether the type of infection was "deep" or "superficial" apparently made no difference in the titer response.

Data have been presented (Figure 2) that indicate a smaller though definite response in antistreptolysin titer among patients with the nephrotic stage of chronic glomerulonephritis than among patients without edema. Previously Longcope (3) had noted that among his 19 cases of "type B" glomerulonephritis, characterized by nephrotic edema, the antistreptolysin titer was rarely above normal and was sometimes persistently very low. A severe hemolytic streptococcal tonsillitis in one of Longcope's nephrotic patients was followed by a rise in antistreptolysin titer that reached a maximum of only 100 units. As Longcope pointed out, the explanation for the low titers and small antistreptolysin response to hemolytic streptococcal infection in these patients is not clear.

In this connection, a recent study (9) has shown that the antistreptolysin titer of children with nephrosis is extremely low, usually less than 10 units, and that when the patient recovers the titer rises to the generally accepted normal range. Furthermore, when these individuals contract group A hemolytic streptococcal infections during the active phase of the nephrosis, in spite of very low base line titers, large rises in antistreptolysin titer ensue in many instances.

SUMMARY AND CONCLUSIONS

1. The serum antistreptolysin titer response was analysed in 81 cases of chronic glomerulonephritis, studied over periods of from 4 months to 8 years.

2. Rises in serum antistreptolysin titer occurring in patients with chronic glomerulonephritis

were of value in detecting the hemolytic streptococcal etiology of many infections in which group A hemolytic streptococci could not be isolated from the pharynx.

3. A number of instances of chronic upper respiratory infection were observed in which repeated cultures showed the presence of group A hemolytic streptococci. These instances unassociated with rises in antistreptolysin titer were designated as the "carrier state."

4. Certain factors affecting the antistreptolysin titer response in chronic glomerulonephritis were examined with the following results:

- a. Sex had no effect.
- b. In general, children exhibited greater titer response to infection than did adults.
- c. The character of the preceding hemolytic streptococcal infection ("deep" or "superficial") did not appear to affect the magnitude of the antistreptolysin titer response.
- d. In general, patients with "nephrotic" edema exhibited smaller rises in titer than did those without edema.

5. The relation of the serum antistreptolysin titer response to the exacerbation in chronic glomerulonephritis is discussed in the accompanying paper (10).

The authors are indebted to Miss Grace Davis and Mr. Walter Meyer for their technical assistance.

BIBLIOGRAPHY

1. Seegal, D., and Lyttle, J. D., Antistreptolysin titer of the serum in acute glomerulonephritis. *Proc. Soc. Exper. Biol. and Med.*, 1933, 31, 211.
2. Lyttle, J. D., Seegal, D., Loeb, E. N., and Jost, E. L.,

The serum antistreptolysin titer in acute glomerulonephritis. *J. Clin. Invest.*, 1938, 17, 631.

3. Longcope, W. T., Studies of the variations in the antistreptolysin titer of blood serum from patients with hemorrhagic nephritis. II. Observations on patients suffering from streptococcal infections, rheumatic fever, and acute and chronic hemorrhagic nephritis. *J. Clin. Invest.*, 1936, 15, 277.
4. Winkenwerder, W. L., McLeod, N., and Baker, M., Infection and hemorrhagic nephritis. *Arch. Int. Med.*, 1935, 56, 297.
5. Todd, E. W., Antihæmolytic titres in hæmolytic streptococcal infections and their significance in rheumatic fever. *Brit. J. Exper. Path.*, 1932, 13, 248.
6. Mote, J. R., and Jones, T. D., Studies of hemolytic streptococcal antibodies in control groups, rheumatic fever, and rheumatoid arthritis. I. The incidence of antistreptolysin "O," antifibrinolysin, and hemolytic streptococcal precipitating antibodies in the sera of urban control groups. II. The frequency of antistreptolysin "O," antifibrinolysin, and precipitating-antibody responses in scarlet fever, hemolytic streptococcal infections, and rheumatic fever. III. The magnitude of antistreptolysin "O," antifibrinolysin, and precipitating-antibody responses; the persistence of the antibodies, and variations in antistreptolysin "O" curves in scarlet fever, hemolytic streptococcal infections, and rheumatic fever. *J. Immunol.*, 1941, 41, 35.
7. Lippard, V. W., and Johnson, P., Beta hemolytic streptococcal infection in infancy and in childhood. I. Antifibrinolysin and antistreptolysin response. *Am. J. Dis. Child.*, 1935, 49, 1411.
8. Bunim, J. J., and McEwen, C., The antistreptolysin titer in rheumatic fever, arthritis and other diseases. *J. Clin. Invest.*, 1940, 19, 75.
9. Lyttle, J. D., Seegal, D., Loeb, E. N., and Jost, E. L., The antistreptolysin titer in nephrosis. Unpublished observations.
10. Earle, D. P., Jr., Seegal, D., Lyttle, J. D., Loeb, E. N., and Jost, E. L., The relation of the serum antistreptolysin titer to the exacerbation in chronic glomerulonephritis. *J. Clin. Invest.*, 1942, 21, 491.

THE RELATION OF THE SERUM ANTISTREPTOLYSIN TITER TO THE EXACERBATION IN CHRONIC GLOMERULONEPHRITIS

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In the present study, the relation of the serum antistreptolysin titer to the exacerbation in chronic glomerulonephritis has been analysed. In a previous study (1), an exacerbation in chronic glomerulonephritis was defined arbitrarily as an abrupt and marked increase in the degree of hematuria. It was pointed out that slight variations in the urinary output of erythrocytes perhaps do not signify the presence of an exacerbation. Exacerbations were frequently associated with some impairment of renal function, generally transient in character. Twenty-eight such episodes were reported in an analysis of 68 cases of chronic glomerulonephritis. The present study is based on this material, with the addition of 5 exacerbations that have been observed since the completion of the earlier study. Two of these exacerbations occurred in a patient (Case XI, PA) previously reported (1), while the other 3 were observed in 2 of 13 new cases studied.

The 33 exacerbations in chronic glomerulonephritis have been observed in 15 of 81 cases, studied over periods of 4 months to 8 years. Each exacerbation was preceded by an upper respiratory tract infection. Group A hemolytic streptococci were recovered by throat culture from 17 of these instances. Adequately studied rises in antistreptolysin titer, as previously defined (2), were associated with 20 of these exacerbations. Definite rises in titer were associated with 4 additional exacerbations, but the data were not sufficient to determine the maximum titer of the rise and therefore not considered in this study.

Six exacerbations were not associated with rises in antistreptolysin titer. In each of these instances determinations were done with sufficient frequency to rule out the possibility of a rise. Group A hemolytic streptococci were recovered by throat culture in one of these instances, hemolytic streptococci whose group was not determined in

another, while the other 4 instances followed infections not proven to be due to the hemolytic streptococcus (head colds in 2, pneumococcus type XXIII otitis media in 1, and lobar pneumonia, causative organism unknown, in 1).

Finally, in 3 instances of exacerbation, determinations were not done with sufficient frequency to discover the presence of a possible brief rise in antistreptolysin titer. Hemolytic streptococci were not recovered by throat culture from any of these 3 instances, the associated infections being head colds, "grippe," and pneumococcus type II ethmoiditis. These instances are not included in the present study.

During the 4 months to 8 year period of observation on these 81 cases of chronic glomerulonephritis, 76 adequately studied rises in antistreptolysin titer were observed in which urinalyses yielded sufficient data to determine the presence or absence of associated exacerbation in chronic glomerulonephritis. The present study does not include 9 of the 85 adequately studied rises in antistreptolysin titer¹ reported in the preceding paper (2).

In summary, the data from which this paper is compiled were obtained from 81 cases of chronic glomerulonephritis in which 76 rises in antistreptolysin titer and 26 exacerbations in chronic glo-

¹ These rises in titer were eliminated from this study for the following reasons: (1) Although rises in titer were associated with sharp increases in the degree of hematuria, the numbers of erythrocytes excreted in the urine during control periods were so variable that it was impossible to determine whether an exacerbation was present or not (4 instances); (2) Hematuria followed an intravenous injection of hemolytic streptococcus nucleoprotein (1 instance); (3) The rise in titer occurred too close to the time of death to permit the development of an exacerbation (3 instances); and (4) The rise in titer followed an unobserved episode that may or may not have been an exacerbation (1 instance).

merulonephritis were both adequately studied. Thus, a correlation or lack of correlation between the two reactions could be determined.

RELATION OF THE MAGNITUDE OF ANTISTREPTOLYSIN TITER RESPONSE TO THE OCCURRENCE OF EXACERBATION IN CHRONIC GLOMERULONEPHRITIS

The 76 rises in antistreptolysin titer in this series were analysed for the presence or absence of exacerbation in chronic glomerulonephritis. It was noted that only rises that attained a maximum titer of 144 units or more were associated with

exacerbations. Further, 13 of the 15 rises that attained a maximum titer of 333 units or more were associated with exacerbations. These points are graphically presented in Figure 1 where it may be seen that the higher the maximum titer of the rise, the more frequent is the occurrence of exacerbation. This figure also shows a similar although not so striking correlation between the magnitude of the rise above the base-line values and the presence or absence of exacerbation.

It should be emphasized once more that 6 exacerbations were not associated with rises in antistreptolysin titer. However, in only one of these

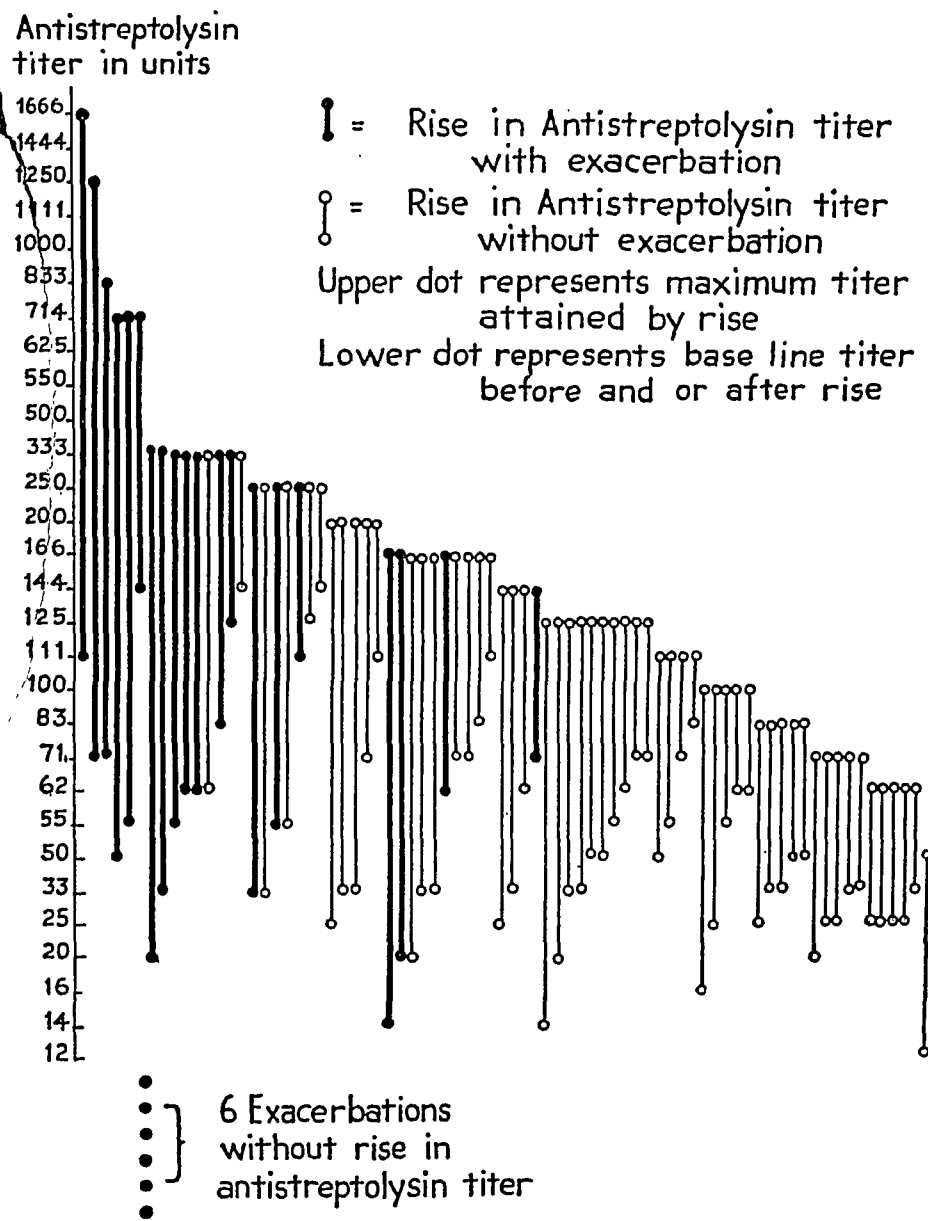


FIG. 1. RELATION OF THE MAXIMUM TITER ATTAINED BY 76 RISES IN ANTISTREPTOLYSIN AND THE MAGNITUDE OF THE RISES TO THE OCCURRENCE OF EXACERBATION IN CHRONIC GLOMERULONEPHRITIS

instances was the preceding infection proven to be due to Group A hemolytic streptococci.

As previously indicated (1), the preponderance of observed exacerbations in chronic glomerulonephritis occurred in children. Furthermore, it has been shown (2) that rises in antistreptolysin titer tended to be greater among the younger patients suffering from chronic glomerulonephritis than among the adults. Thus, both exacerbations and large rises in antistreptolysin titer were uncommon among adults. However, 3 of the 6 rises attaining a maximum titer of 250 units or more that were observed in patients 20 years of age or older were associated with exacerbations. These relations are shown in Figure 2. It seems likely, therefore, that there is a more direct correlation

between the magnitude of the antistreptolysin titer response and the incidence of exacerbations than between age and the incidence of exacerbations.

In the preceding paper (2) it was pointed out that the antistreptolysin titer response was, in general, greater among patients with chronic glomerulonephritis without nephrotic edema than among patients with edema. Thus, 19 of the 50 rises observed among patients without the nephrotic element attained a maximum titer of 250 units or more, while only 6 of the 35 rises found in edematous patients reached similar levels. However, the incidence of exacerbations associated with rises of this magnitude was approximately the same in patients without and with edema, 13 of 17 and 3 of 5 respectively (disregarding the several rises associated with equivocal exacerbations). Thus, there appears to be a more direct correlation between the magnitude of the antistreptolysin titer response and the incidence of exacerbations, than between the status of the nephritis (with or without nephrotic edema) and the occurrence of exacerbations.

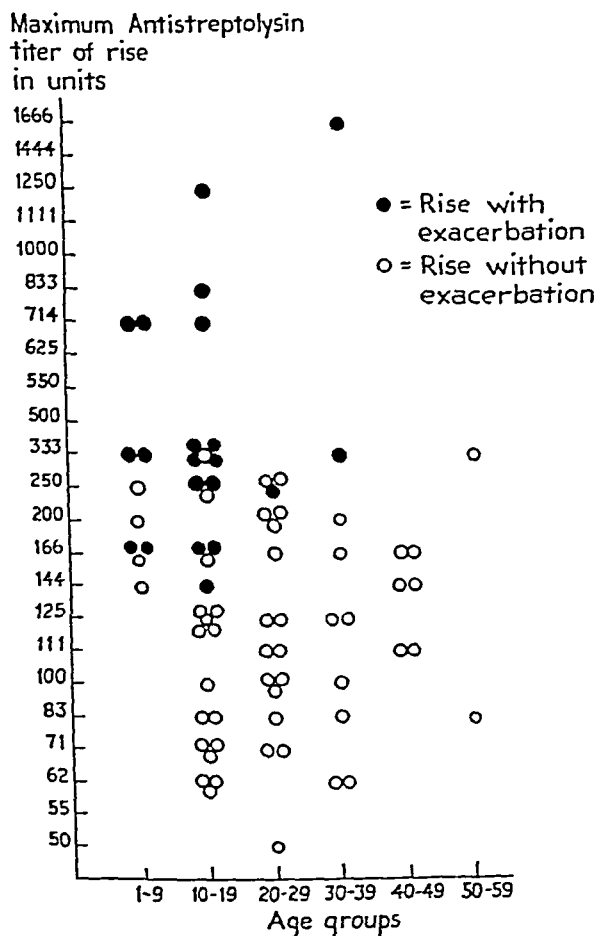


FIG. 2. RELATION OF THE OCCURRENCE OF EXACERBATION IN CHRONIC GLOMERULONEPHRITIS TO THE MAXIMA OF RISES IN ANTISTREPTOLYSIN TITER AND TO AGE

RELATION OF THE ONSET OF ANTISTREPTOLYSIN TITER RISE TO THE ONSET OF EXACERBATION IN CHRONIC GLOMERULONEPHRITIS

In 8 instances of exacerbation, antistreptolysin titer determinations were done at intervals that permitted conclusions as to whether the exacerbation began before, during, or after the onset of the rise in titer. These 8 instances are shown in Figure 3, where the onset of infection is represented by the heavy vertical line. Control antistreptolysin titer values are shown to the left of this line, while the rises in titer are on the right. The onset of hematuria in each instance is indicated by an arrow.

It may be noted in Figure 3 that the exacerbation occurred before the rise in antistreptolysin titer was apparent in 7 of these 8 instances. This is not surprising in view of the brief latent period between the preceding infection and the onset of the exacerbation in chronic glomerulonephritis (3). In addition, each of the 20 exacerbations associated with adequately studied rises in antistreptolysin titer occurred well before the peak of the rise was reached.

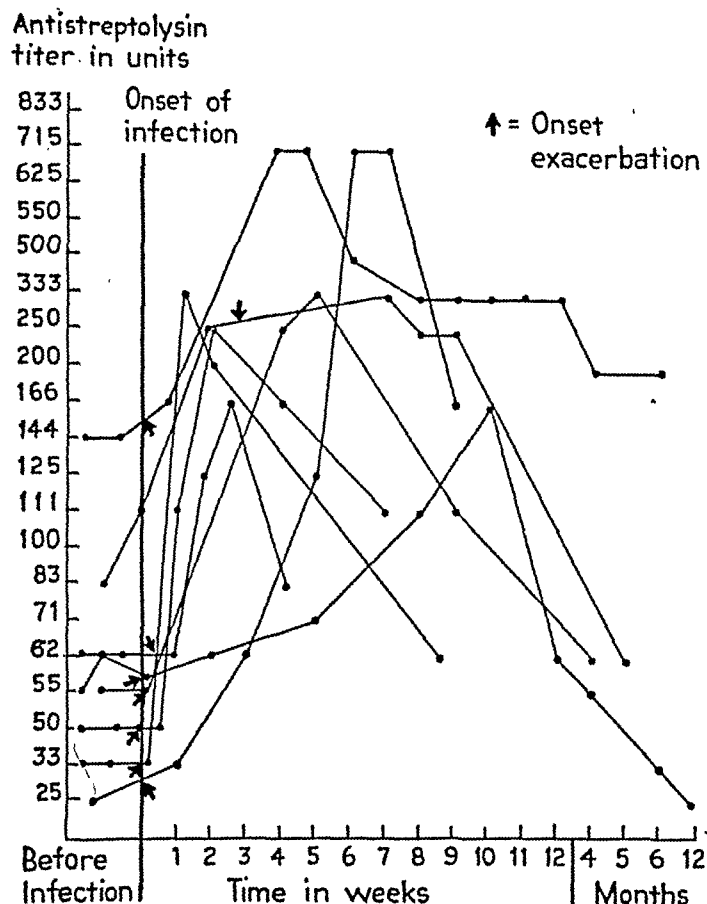


FIG. 3. RELATION OF ONSET OF RISE IN ANTISTREPTOLYSIN TITER TO ONSET OF EXACERBATION IN CHRONIC GLOMERULONEPHRITIS

RELATION OF THE ANTISTREPTOLYSIN TITER RESPONSE TO IMPAIRMENT OF RENAL FUNCTION DURING EXACERBATION IN CHRONIC GLOMERULONEPHRITIS

The renal function was investigated before, during, and after the exacerbations as previously described (1) by means of either the urea clearance test, the phenolsulphonphthalein excretion test, the urea ratio of Mosenthal and Bruger (4), or the serum non-protein nitrogen level, or by a combination of these. As pointed out in the earlier study of exacerbations in chronic glomerulonephritis (1), the inability to demonstrate a decrease in renal function following certain exacerbations may be related either to the slight degree of the renal damage or to failure to employ appropriate renal function tests at sufficiently frequent intervals.

However, some impairment of renal function was demonstrated by one or more of the tests mentioned above in 21 of the 33 exacerbations²

² Protocols of 28 of these exacerbations have been published (1).

under consideration in this study. The impairment in function was transient with but one exception (Case X, ED) (1).

The significance of this transient impairment of renal function in the exacerbation in chronic glomerulonephritis is not certain. Goldring (5, 6) has shown, using the urea clearance test, that during the acute stage of rheumatic fever and lobar pneumonia there may be renal hyperfunction followed by transient depression of function. Since the exacerbation in chronic glomerulonephritis occurs so soon after the preceding infection, there is the possibility that the transient impairment of renal function observed in many exacerbations in chronic glomerulonephritis may in part represent a peculiar response to infection.

Seventeen of the 24 exacerbations associated with definite rises in antistreptolysin titer showed some evidence of renal function impairment. Similarly, 4 of the 6 exacerbations, in which adequate antistreptolysin titer studies revealed no rises, were associated with impairment of renal function. Thus, the presence or absence of associated rises in titer did not appear to modify the incidence of renal function impairment (as measured in this study) in exacerbations in chronic glomerulonephritis.

DISCUSSION

As part of a study on the effects of upper respiratory infection on the course of chronic glomerulonephritis, the relation of the serum antistreptolysin titer to the exacerbation in this disease has been investigated. In a previous report (1), it was indicated that each exacerbation observed in chronic glomerulonephritis was preceded by an infection, generally due to the group A hemolytic streptococcus. It was also noted that a transient decrease in renal function was a common feature in exacerbations in chronic glomerulonephritis. In an effort to obtain further data on the mechanism of the development of exacerbations in chronic glomerulonephritis, this analysis of the relation between the serum antistreptolysin titer response and the exacerbation was undertaken.

The data presented above indicate that when patients suffering from chronic glomerulonephritis develop rises in antistreptolysin titer following Group A hemolytic streptococcal infections, the

incidence of exacerbations is greater among the instances of large rises in titer than among the small rises. This finding does not indicate that a rise in antistreptolysin titer is a prerequisite for the development of an exacerbation (as defined above) in chronic glomerulonephritis, since it has been pointed out that the exacerbation usually occurs before the rise in titer is apparent. More important, 6 instances of exacerbation were not associated with rises in titer. Although the lowest maximum value attained by a rise in antistreptolysin titer associated with an exacerbation in the present study was 144 units, Longcope (7) recorded an instance of exacerbation in which the associated rise in titer reached a maximum of only 100 units. However, it does appear that when there is an antistreptolysin titer response, the higher the rise in titer, the more likely is the occurrence of associated exacerbation.

The explanation for this apparent correlation between the magnitude of the antistreptolysin titer response and the occurrence of exacerbations in chronic glomerulonephritis is not clear from the data at hand. However, several possibilities may be considered, all of which have been mentioned by several authors (7, 8, 9, 10, 11, 12, 13) in discussions of the relation of the serum antistreptolysin titer to various diseases due to or associated with the group A hemolytic streptococcus. For example, certain strains of hemolytic streptococci may be more prone to evoke both exacerbations and larger rises in titer than other strains, although there is no direct evidence to support this possibility.

The possibility that exacerbations follow severe infections rather than mild ones and that the associated large rises in titer are a reflection of the severity of the preceding infections is not supported by two observations. First, 14 of the 24 exacerbations associated with rises in titer followed mild infections (pharyngitis 12 instances, tonsillitis 1 instance, and "common cold" 1 instance). Indeed, the two highest rises in antistreptolysin titer (1250 and 1666 units) were associated with exacerbations followed infections of this type. And second, as pointed out in the preceding paper (2), there was no apparent correlation between the magnitude of the antistreptolysin titer response in chronic glomerulonephritis

and the "deepness" or "superficiality" of the preceding infection. The superficial infections include pharyngitis, tonsillitis and "common cold." Mote and Jones (13) likewise state, ". . . in both the mild and the severe infections every type of antistreptolysin 'O' curve has been encountered."

The association of exacerbations with large rather than small rises in antistreptolysin titer might be explained by an increase in the ability of chronic nephritics undergoing exacerbation to produce antistreptolysin in response to group A hemolytic streptococcal infections. If this be so, however, it is not clear whether the development of an exacerbation depends on an altered antibody response, whether the reverse is true, or whether both reactions are simply two manifestations of some other process.

There have been some observations that support the view that, in certain diseases due to or associated with group A hemolytic streptococci, the antibody response to the infection differs from the response to uncomplicated infections. Thus, Curn and Pauli (11) found that attacks of acute tonsillitis and pharyngitis in "normal" individuals were infrequently followed by rises in antistreptolysin titer, and that when rises did occur they rarely reached high values. In contrast, increases in antistreptolysin were more constant and greater in magnitude after similar infections associated with the development of attacks of rheumatic fever. Longcope (7) made similar observations and suggested that, "It might be found that the response of this particular antibody is exaggerated in erysipelas, scarlatina, rheumatic fever and in one form of acute hemorrhagic nephritis." Mote and Jones (13), however, in the summary of their extensive studies on hemolytic streptococcal antibodies in 1336 control cases, 399 cases of hemolytic streptococcal infections, 749 cases of rheumatic fever, and 285 cases of various types of infections, stated: "In our opinion, none of the analyses, including a detailed examination of the antistreptolysin 'O' curves in both rheumatic and non-rheumatic subjects, have revealed any basic difference in the hemolytic streptococcal antibody-response, insofar as the antibodies investigated are concerned, between the non-rheumatic and the rheumatic individual in their reaction to infection by the hemolytic streptococcus."

In the present study, although there was a definite correlation between the magnitude of the antistreptolysin titer response (when present) to the incidence of exacerbation in chronic glomerulonephritis, 6 exacerbations were not associated with a rise in titer. Two of these followed hemolytic streptococcal infections and probably represent definite exceptions to the usual association of a rise in antistreptolysin titer, in response to infection, and the development of an exacerbation. However, the other 4 exacerbations, not associated with rises, followed infections not shown to be of hemolytic streptococcal origin. These may possibly have represented some non-specific reaction of the kidneys.

It has been noted that the onset of the exacerbation generally precedes the onset of the rise in serum antistreptolysin titer. This is in striking contrast to the findings of Coburn (14) in regard to rheumatic fever. This author found that attacks of rheumatism usually developed when the titer was still rising, in the second week after the preceding infection. This may represent a real difference between the immune responses of patients with glomerulonephritis and with rheumatic fever, and appears similar to other biological differences between these conditions, discussed in an earlier communication (3).

SUMMARY AND CONCLUSIONS

1. Thirty-three exacerbations in chronic glomerulonephritis have been observed in 15 of 81 nephritic patients, studied for from 4 months to 8 years. Each exacerbation was preceded by an infection.

2. Twenty-four of these exacerbations were associated with rises in serum antistreptolysin titer, in 6 there was no rise, and in 3 the data were insufficient to determine whether a rise in titer had occurred.

3. When it occurred, the greater the magnitude of the rise in antistreptolysin titer, the greater was the incidence of associated exacerbation in chronic glomerulonephritis.

4. The exacerbation preceded the onset of the rise in antistreptolysin titer in 7 of the 8 sufficiently studied instances.

5. There was a high incidence of transient im-

pairment of renal function in exacerbation associated with and also without rises in antistreptolysin titer.

The authors are indebted to Miss Grace Davis and Mr. Walter Meyer for their technical assistance.

BIBLIOGRAPHY

1. Seegal, D., Lyttle, J. D., Loeb, E. N., Jost, E. L., and Davis, G., On the exacerbation in chronic glomerulonephritis. *J. Clin. Invest.*, 1940, 19, 569.
2. Earle, D. P., Jr., Loeb, E. N., Seegal, D., Lyttle, J. D., and Jost, E. L., The serum antistreptolysin titer in chronic glomerulonephritis. *J. Clin. Invest.*, 1941, 21, 483.
3. Seegal, D., and Earle, D. P., Jr., A consideration of certain biological differences between glomerulonephritis and rheumatic fever. *Am. J. M. Sci.*, 1941, 201, 528.
4. Mosenthal, H. O., and Bruger, M., The urea ratio as a measure of renal function. *Arch. Int. Med.*, 1935, 55, 411.
5. Goldring, W., Studies of the kidney in acute infection. II. Observations with the urea clearance test in acute rheumatic infection. *J. Clin. Invest.*, 1931, 10, 345.
6. Goldring, W., Studies of the kidney in acute infection. III. Observations with the urine sediment count (Addis) and the urea clearance test in lobar pneumonia. *J. Clin. Invest.*, 1931, 10, 355.
7. Longcope, W. T., Studies of variations in antistreptolysin titer of blood serum from patients with hemorrhagic nephritis; observations on patients suffering from streptococcal infections, rheumatic fever, and acute and chronic hemorrhagic nephritis. *J. Clin. Invest.*, 1936, 15, 277.
8. Coburn, A. F., and Pauli, R. H., Studies on the relationship of *Streptococcus hemolyticus* to the rheumatic process. III. Observations on the immunological responses of rheumatic subjects to hemolytic streptococcus. *J. Exper. Med.*, 1932, 56, 651.
9. Myers, W. K., and Keefer, C. S., Antistreptolysin content of the blood serum in rheumatic fever and rheumatoid arthritis. *J. Clin. Invest.*, 1934, 13, 155.
10. Wilson, M. G., Wheeler, G. W., and Leask, M. M., The relation of upper respiratory infections to rheumatic fever in children. II. Antihemolysin titers in respiratory infections and their significance in rheumatic fever in children. *J. Clin. Invest.*, 1935, 14, 333.
11. Coburn, A. F., and Pauli, R. H., Studies on the immune response of the rheumatic subject and its relationship to activity of the rheumatic process. VI. The significance of the rise of antistreptolysin level in the development of rheumatic activity. *J. Clin. Invest.*, 1935, 14, 769.

12. Bunim, J. J., and McEwen, C., The antistreptolysin titer in rheumatic fever, arthritis, and other diseases. *J. Clin. Invest.*, 1940, 19, 75.
13. Mote, J. R., and Jones, T. D., Studies of hemolytic streptococcal antibodies in control groups, rheumatic fever, and rheumatoid arthritis. I. The incidence of antistreptolysin "O," antifibrinolysin, and hemolytic streptococcal precipitating antibodies in the sera of urban control groups. II. The frequency of antistreptolysin "O," antifibrinolysin, and precipitating-antibody responses in scarlet fever, hemolytic streptococcal infections, and rheumatic fever. III. The magnitude of antistreptolysin "O," antifibrinolysin, and precipitating-antibody responses; the persistence of the antibodies, and variations in antistreptolysin "O" curves in scarlet fever, hemolytic streptococcal infections, and rheumatic fever. *J. Immunol.*, 1941, 41, 35.
14. Coburn, A. F., Faulty disposal of *Streptococcus hemolyticus* in relation to the development of the rheumatic lesion. *Tr. and Stud., Coll. Physicians, Philadelphia*, 1940, 8, 91.

THE UREA CLEARANCE OF YOUNG PREMATURE AND FULL TERM INFANTS^{1,2}

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In a previous study (1) of the nitrogen metabolism of premature infants, it was noted that edema developed in some infants when they were changed from diets of human milk to diets of cow's milk containing equivalent amounts of protein, fat, carbohydrate, and fluid. In subsequent studies of water and electrolyte exchange under similar dietary conditions (2), a lag in renal excretion of the extra sodium and chloride ingested in the cow's milk was found to accompany the large gain in weight. These observations suggested the present study to determine whether premature infants have a defect in kidney function. This seemed particularly desirable since the adequacy of renal function (3) presumably acts as a major determinant of minimal fluid requirements.

As a first approach to this problem, measurements were made of the 24-hour plasma urea clearance in a group of young prematurely born and full term infants. The results reported in this paper indicate that the urea clearance of premature infants is lower than that of full term infants of comparable postnatal age, and that in both groups the clearance is lower than that reported for older infants and adults. Moreover, the urea clearance of individual infants rose strikingly with increasing age. No relation was found to exist between urea clearance and urine flow for the group as a whole, and in each of 4 infants, augmentation of the urine flow from 25 to 100 per cent by increasing the fluid intake did not improve the urea clearance.

METHODS

Subjects

Fifteen healthy, male premature infants, ranging in age from 8 to 65 days and in weight from 1.6 to 3.4 kgm.,

¹ Assistance in this work was given by the Children's Bureau, U. S. Department of Labor.

² Presented in part before the meeting of the Society for Pediatric Research, Atlantic City, N. J., May 7, 1941. (*Am. J. Dis. Child.*, 1941, 62, 894.)

were studied in 35 observations. Twelve observations were made in 9 male full term infants whose ages and weights ranged from 7 to 73 days, and from 2.8 to 4.9 kgm., respectively. All the infants were thriving at the time of study.

Diets

In all but one observation, the diets consisted of cow's milk³ diluted with water and fortified in most instances with either cane sugar or dextrimaltose to provide an adequate caloric intake. In all but 4 observations (Tables II and III), the daily protein intake was high, *i.e.* from 4 to 6 grams per kgm. The daily fluid intake was within the customary range of 130 to 185 cc. per kgm. in all but 3 observations. All the infants received 10 or 20 drops daily of a vitamin A and D concentrate (percomorph oil); approximately half received daily supplements of 25 mgm. of ascorbic acid.

Urine and blood

Urine was collected (4), using toluol as a preservative, for carefully timed periods of approximately 24 hours. One-half to 1 cc. of venous or occasionally capillary blood was drawn into short tubes, containing either oxalate or heparin, at the beginning and end of the 24-hour period. Blood urea nitrogen was determined gasometrically, using the method of Van Slyke and Kugel (5). Urine urea and ammonia nitrogen were similarly determined and calculations of urea excretion made on the assumption that the ammonia appearing in the urine had been filtered through the glomeruli as urea. This was considered a fairer estimate of urea excretion (6) than the determination of urea alone since, in addition to the variable formation of ammonia from urea by the renal tubules, bacterial formation of ammonia in voided urine was not consistently prevented.⁴

The 24-hour clearance method used by Landis and his co-workers (7) is particularly suited to young infants for two reasons. Catheterization and washing out the bladder

³ The following preparations of cow's milk were used: evaporated or powdered whole milk, powdered half-skimmed milk (alacta), a powdered skimmed milk—olive oil preparation (olac), and a half-skimmed olac. The latter product was prepared specially by the Mead Johnson Co.

⁴ Urinary ammonia averaged 14 per cent of the total urinary urea plus ammonia and was less than 20 per cent in 34 of 40 observations in which the partition was determined (Tables II and III).

to insure complete emptying may be omitted without introducing a large error, since the amount of urine retained by spontaneous incomplete voidings comprises only a relatively small fraction of the 24-hour urine flow (volume 75 to 400 cc. per 24 hours in these observations). Secondly, the feeding to these infants of their daily diets in 6 or 8 aliquots at 4 or 3 hour intervals tends to minimize fluctuations in blood urea (Table I).

TABLE I
Hourly variations in blood urea nitrogen

Hours* after feeding	Blood urea nitrogen							Average	Maximum devia- tion
	1	1½	2	2½	3	3½	4		
<i>Premature</i>	<i>mgm. per 100 cc.</i>								<i>per cent average</i>
T. R.		23.7		27.1		23.8		24.9	9
J. S.	33.4		30.9		31.8		30.4	31.6	6
C. M.		17.0		17.5				17.3	2
C. T.	27.3		29.6	27.4				28.1	5
I. A.	25.9		26.1		24.0			25.3	8
<i>Full term</i>									
A. A.	20.4			20.9			20.1	20.5	2
F. M.	19.1			17.9		18.4		18.5	3
W. C.			20.1		20.7	20.8		20.5	2
T. T.					27.4 31.2		29.8	29.5	7
J. R.					26.9 28.3		27.8	27.7	3
K. K.	17.5		22.0		16.1		17.5	18.3	20
C. A.			17.9	19.3	19.8	19.9	19.9 18.5	19.2	7

* Infants were fed every 3 or 4 hours throughout the 24 hours, and blood was drawn at the stated intervals following meals.

For each of 5 premature and 7 full term infants, urea nitrogen was determined in 3 or 4 samples of blood taken from 1 to 4 hours following the same or a similar feeding at different times of the day. The maximum deviation in any one specimen was, for 11 of the 12 infants, between 2 and 9 per cent of the average. In 1 infant (K. K.) the maximum deviation in a single specimen was 20 per cent of the average, but omission of this determination would have changed the average blood urea by only 8 per cent. It is of interest that the blood urea nitrogen of this group of thriving infants fed high protein diets (see also Tables II and III) is considerably higher than the customary levels found in adults.

RESULTS

The detailed results of the observations on premature and full term infants are presented in Tables II and III, respectively, and a summary of the results in Table IV.

TABLE II
Urea clearance of prematurely born infants

Subject	Age	Weight	Surface area(a)	Urine		Blood urea nitro- gen	Urea clearance cc. per minute	
				Vol- ume	Urea and am- monia nitro- gen(b)			
	<i>days</i>	<i>grams</i>	<i>sq. meter</i>	<i>cc. per minute</i>	<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>	<i>UV/B</i>	<i>per sq. meter</i>
F. L.	17	1600	0.131	0.112	54	5.4(c)	1.1	8.5
	65	3205	0.221	0.259	64	6.5(c)	2.5	11.5
R. K.	27	1800	0.143	0.052	859	26.4	1.7	11.8
	30	1915	0.150	0.096	464	23.2	1.8	11.9
H. A.	10	1890	0.149	0.131	314	23.9	1.7	11.4
	29	2520	0.184	0.183	464	22.5	3.8	20.7
	50	3630	0.243	0.254	371	19.8	4.8	19.8
W. B.	19	1930	0.151	0.132	350	23.5	2.0	13.2
	23	1990	0.155	0.139	286	22.1	1.8	11.6
	24	2010	0.156	0.133	342	20.8	2.2	14.1
	40	2710	0.195	0.110	437	17.6	2.7	13.8
	47	2970	0.209	0.174	461	20.2	4.0	19.1
E. D.	31	1953	0.152	0.064	830	21.9	2.4	15.8
A. G.	23	1970	0.153	0.106	346	19.9	1.8	11.8
	24	2040	0.158	0.127	283	21.1	1.7	10.8
	35	2445	0.180	0.135	370	19.0	2.6	14.4
B. G.	18	1985	0.154	0.126	200	31.0	1.6	10.5
	23	2170	0.165	0.140	392	25.7	2.1	12.7
	31	2510	0.184	0.173	334	19.3	3.0	16.3
P. A.	10	2125	0.162	0.116	429	26.1	1.9	11.7
	29	2750	0.197	0.195	547	32.7	3.3	16.8
	31	2800	0.200	0.249	449	31.2	3.6	17.9
	45	3410	0.232	0.242	523	27.1	4.7	20.3
J. S.	19	2160	0.164	0.106	434	23.2	2.0	12.2
J. C.	8	2200	0.167	0.133	347	22.4	2.1	12.6
	14	2360	0.176	0.141	99	5.2(c)	2.7	15.3
	43	3260	0.224	0.248	436	21.3	5.1	22.8
T. R.	21	2209	0.167	0.080	724	26.6	2.2	13.2
	49	3035	0.212	0.302	391	23.1	5.1	24.1
	58	3340	0.228	0.208	656	24.9	5.5	24.1
V. C.	8	2393	0.178	0.144	612	32.4	2.7	15.3
	10	2385	0.177	0.230	322	27.4	2.7	15.3
R. L.	27	2420	0.179	0.183	447	27.5	3.0	16.8
R. DeL.	32	2555	0.186	0.226	157	13.4	2.6	14.2
A. H.	23	2965	0.208	0.139	674	21.2	4.4	21.3

(a) Surface area for both premature and full term infants was calculated using the formula $5.188 \times \text{wt.}^{.75}$ (8).

(b) Urea plus ammonia nitrogen was approximately 90 per cent of the total urinary nitrogen.

(c) These infants received a low protein diet.

In Table IV, the observations have been divided into two groups according to postnatal age. For both groups, under and over 30 days of age, the clearance was lower in premature than in full term infants. The mean clearance for the whole group of premature infants was 15.3 cc. per sq. meter per minute, as compared with 21.0 cc. for the full term infants. Although the ranges overlap, the difference of 5.7 cc. between the mean clearances is almost five times the probable error

TABLE III
Urea clearance of full term infants

Subject	Age	Weight	Sur- face area	Urine		Blood urea nitrogen	Urea clearance cc. per minute	
				Vol- ume	Urea and am- monia ni- trogen		UV/B	per sq. meter
	days	grams	sq. meter	cc. per minute	mgm. per 100 cc.	mgm. per 100 cc.		
R. J.	30	2790	0.199	0.220	398	23.8	3.7	18.5
W. C.	7	3255	0.224	0.125	246	10.0*	3.1	13.8
	27	3700	0.246	0.146	648	20.5	4.6	18.7
J. R.	39	3730	0.248	0.229	660	27.8	5.4	21.7
W. D.	14	4163	0.269	0.136	741	17.0	5.9	22.0
K. K.	71	4200	0.271	0.136	1049	18.3	7.8	28.8
	73	4275	0.274	0.270	515	16.2	8.6	31.3
D. S.	59	4280	0.275	0.256	507	19.3	6.7	24.5
T. T.	27	4360	0.278	0.191	748	30.5	4.7	16.8
	41	4860	0.302	0.188	691	25.7	5.1	16.7
G. M.	54	4530	0.286	0.156	689	15.2	7.1	24.7
V. F.	23	4600	0.290	0.099	662	16.1	4.1	14.1

* This infant received a relatively low protein intake (3 grams per kgm.).

TABLE IV
Urea clearance of premature and full term infants.
Summary of results

Postnatal age	Premature infants			Full term infants		
	Num- ber of ob- serva- tions	Urea clearance		Num- ber of ob- serva- tions	Urea clearance	
		Mean	Range		Mean	Range
		cc. per sq. meter per minute			cc. per sq. meter per minute	
Less than 30 days...	21	13.7	8.5-21.3	6	17.3	13.8-22.0
30 days and over...	14	17.6	11.5-24.1	6	24.6	16.7-31.3
Total.....	35	15.3*	8.5-24.1	12	21.0*	13.8-31.3
		P.E. 0.47			P.E. 1.08	

* Difference between means is 5.7 ± 1.18 .

of the difference. The mean clearances for both groups of young infants (15 and 21 cc.) are considerably lower than the average clearance of 38 cc. per sq. meter per minute (range 23 to 55 cc.) found in older infants by Schoenthal (9), and the "standard" and "maximum" clearances of adults, 30 and 40 cc. per sq. meter per minute (10). Since, as will be shown later, the clear-

ances in these young infants were "maximum" at the time of observation, i.e., increasing the urine flow did not increase the clearance, there can be no doubt that young infants, both premature and full term, have a defect in urea clearance when compared with older infants and adults. Evidence confirming the existence of this defect in young infants has recently been published (11).

Effect of increasing size and postnatal age on urea clearance

The effect of increasing maturation as measured by increasing size and age on urea clearance is indicated in Figure 1, in which repeated observations on 8 premature and 2 full term infants are presented. The ages at the initial and final observations for each infant are also indicated. It is seen that a rise in clearance took place in each of the 8 premature and in 1 of the 2 full term infants as they grew larger and older. The figure also gives some measure of the variability in clearance between infants of similar size as well as age.

Infant F. L. (premature), who showed a low clearance at both 17 and 65 days of age, was the only infant on a low protein diet at the time of both observations. It has been reported (12) that under certain conditions low protein diets depress the urea clearance and this may have contributed to the persistently low findings for this infant. Further work is needed to elucidate this point.

TABLE V
Effect of increased urine flow on 24-hour urea clearance

Subject	Age	Weight	Fluid in- take	Urine			Blood urea nitro- gen	Urea clearance
				Volume		Urea and am- monia ni- trogen		
				cc.	cc. per sq. meter per minute	mgm. per cent	mgm. per cent	cc. per sq. meter per minute
R. K.	27	1800	111	0.05	0.36	859	26.4	11.8
	30	1915	146	0.10	0.64	464	23.2	11.9
V. C.	8	2393	133	0.14	0.81	612	32.4	15.3
	10	2385	218	0.23	1.30	323	27.4	15.3
P. A.	29	2750	145	0.20	0.99	547	32.7	16.6
	31	2800	186	0.25	1.25	449	31.2	17.9
K. K.	71	4200	136	0.14	0.50	1084	18.3	29.8
	73	4275	182	0.27	0.98	515	16.2	31.3

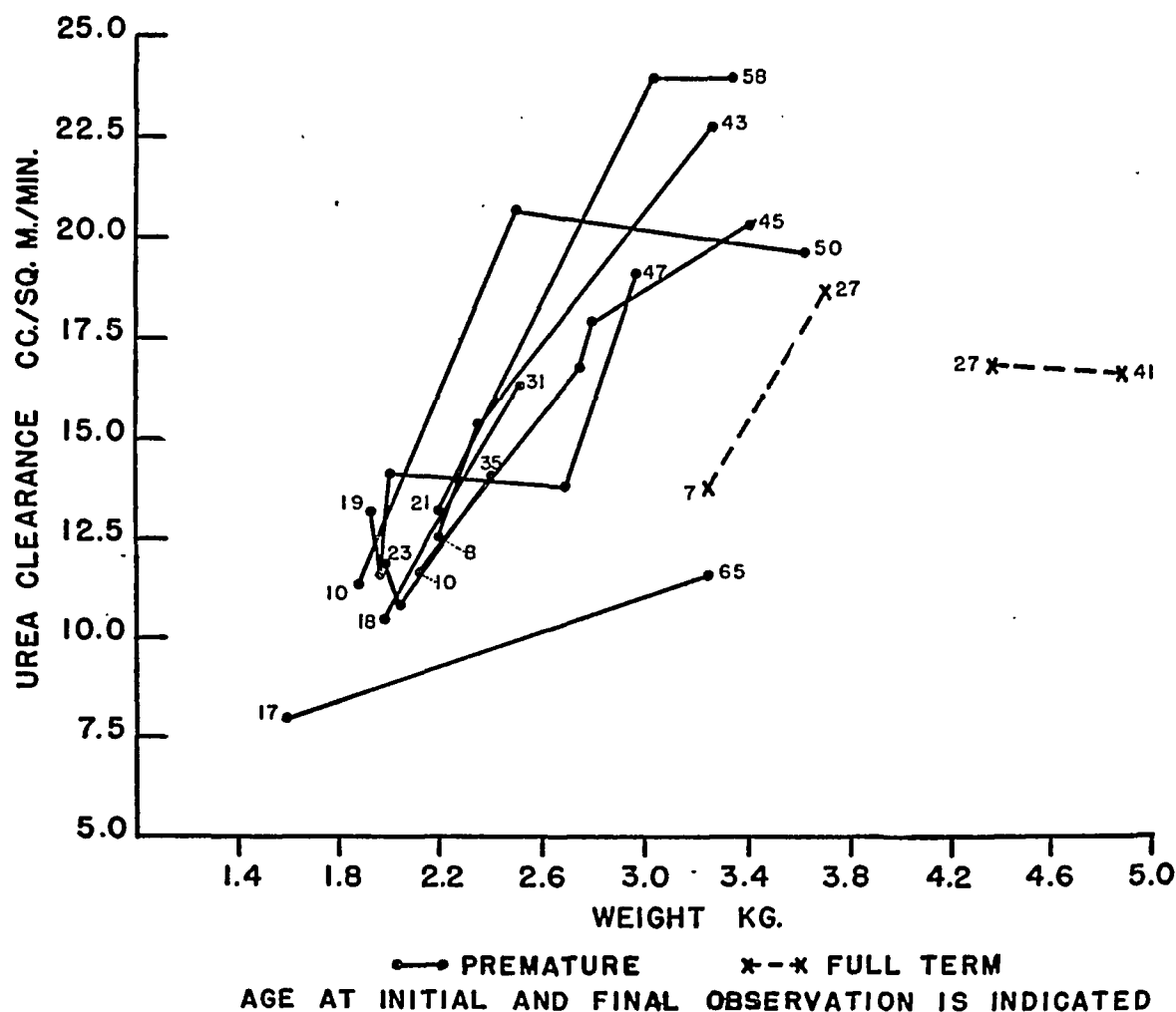


FIG. 1. THE RELATION OF INCREASING SIZE AND DAYS OF AGE TO UREA CLEARANCE IN INDIVIDUAL INFANTS

Effect of variations in urine flow on urea clearance

Because the urine flows for most of the infants were below the arbitrary "augmentation" limit for adults of approximately 0.9 to 1.2 cc. per sq. meter per minute (*i.e.* 1.5 to 2.0 cc. per minute), the effect of increasing the urine flow on the urea clearance was studied in 4 infants. In each of these infants, the urea clearance was determined at two levels of fluid intake, at least 24 hours of the altered intake elapsing before the second observation began. Increasing the fluid intake increased the initial urine flows of 0.4 to 1.0 cc. per sq. meter per minute by from 25 to 100 per cent, but this was accompanied by a proportional decrease in urinary concentration of urea. Since the blood urea did not change greatly, the clearances remained constant. This lack of relation of urine flow, within the ranges studied, to urea clearance is confirmed by the low coefficient of corre-

lation (0.17 ± 0.09) between urea clearance and urine flow computed for the whole group of observations (Tables II and III).

These results are not in accord with the conclusions of McCance and his coworkers (11), who using uncatheterized specimens collected over probably short periods, reported a direct relationship between the urea clearance and the minute volume of urine. They considered this relationship as affording added evidence for instability of renal function in young infants. The discrepancy may be explained in part by the fact that most of the infants studied by McCance and his coworkers were less than 14 days of age, and it may be that in very young infants under conditions of low fluid intakes and very low urine flows (*e.g.*, 0.2 cc. per sq. meter per minute) (11) there is a direct relation between urea clearance and urine flow. The reported results, however, show a large scatter. Furthermore, estimations of urea clearance from

spontaneously voided specimens over short periods permit the introduction of a significant systematic error which would show low urea clearances for low urine volumes and large clearances for large volumes. Either catheterized specimens for short periods of time or long periods of collections such as were used in the present study are necessary to minimize this source of error. Suffice it to say that using the 24-hour method, no relation was found in the present study between urine flow and urea clearance, this lack of correlation being evident in an 8-day-old premature infant and in a full term infant of 70 days, over a range of clearances of from 12 to 30 cc. per sq. meter per minute, and over a range of initial urine flows of from 0.4 to 1.0 cc. per sq. meter per minute.

DISCUSSION

The low urea clearance of young infants may be caused by diminished glomerular filtration or increased tubular reabsorption of urea. Shannon (13) has demonstrated that with low urine flows there is an increased reabsorption of urea, possibly due to prolonged contact of urine with the cells of the reabsorbing tubules. The lack of correlation in the whole group of observations between urine flow and urea clearance, and the lack of effect in 4 infants of sharp rises in urine flow on urea clearance, suggest that increased tubular reabsorption of urea due to low urine flow is not the cause for the low urea clearances observed in this study.

The other explanation for diminished urea clearances is a decreased glomerular filtration. We have not yet had the opportunity to measure simultaneous inulin and urea clearances in young infants, but Barnett (14) has reported that the rate of removal of inulin from the blood is slower in full term infants of 5 to 9 days than in infants of 2 to 7 weeks, and that in both groups the rate is slower than in children of 6 to 10 years. Although, as has been pointed out (15), these data are inadequate for quantitative interpretation of the blood inulin curves in terms of clearance, Barnett's explanation would account for the higher urea clearances in older infants noted by McCance and Young (11a) and those reported in the present study.

Histological studies (16) of the glomeruli of the fetus and newborn infant supply anatomical support for the supposition of defective glomerular filtration. The earlier the stage of development of the kidney, the fewer the rows of glomeruli in the cortex, and the less convoluted the capillaries. The actual amount of blood coursing through the glomeruli and the size of the filtering surface may thus be limited by the state of development of the glomeruli. Another factor limiting filtration is the extent of the layer of cuboidal cells lining the glomerulus. The shedding of this layer may be wholly developmental or it may be partly dependent on postnatal circulatory changes. Finally, glomerular dynamics in these young infants may be conditioned by postnatal physiologic adjustments of a systemic rather than solely renal character.

Studies of inulin and diodrast clearance may throw light on the mechanisms involved. Definition of the relation of defective renal function in young infants to peculiarities in water and acid-base metabolism (17) and to such clinical states as dehydration fever, acidosis and tetany in the newborn period must await completion of studies of tubular function.

SUMMARY

The 24-hour plasma clearance of urea was determined in 35 observations on 15 premature infants and in 12 observations on 9 full term infants, ranging in age from 8 to 65 and from 7 to 73 days, respectively. The urea clearance is lower in premature than in full term infants, and in both groups of young infants it is lower than that reported for older subjects. No relation was found to exist between urine flow and urea clearance.

BIBLIOGRAPHY

1. Gordon, H. H., Levine, S. Z., Wheatley, M. A., and Marples, E., Respiratory metabolism in infancy and in childhood. XX. The nitrogen metabolism in premature infants. Comparative studies of human milk and cow's milk. *Am. J. Dis. Child.*, 1937, 54, 1030.
2. Gordon, H. H., and Levine, S. Z., Unpublished observations.
3. Gamble, J. L., Extracellular fluid: Extracellular fluid and its vicissitudes. *Bull. Johns Hopkins Hosp.*, 1937, 61, 151; Extracellular fluid: Renal defense of extracellular fluid; control of acid-base excre-

- tion and the factors of water expenditure. *Ibid.*, 1937, 61, 174.
4. Hoag, L. A., Apparatus for quantitative collection of urine and of stools in male infants. *Am. J. Dis. Child.*, 1932, 44, 770.
 5. Van Slyke, D. D., and Kugel, V. H., Improvements in manometric micro-kjeldahl and blood urea methods. *J. Biol. Chem.*, 1933, 102, 489.
 6. Van Slyke, D. D., Page, I. H., Hiller, A., and Kirk, E., Studies of urea excretion. IX. Comparison of urea clearances calculated from the excretion of urea, of urea plus ammonia and of nitrogen determinable by hypobromite. *J. Clin. Invest.*, 1935, 14, 901.
 7. Landis, E. M., Elsom, K. A., Bott, P. A., and Shiels, E., Observations on sodium chloride restriction and urea clearance in renal insufficiency. *J. Clin. Invest.*, 1935, 14, 525.
 8. Klein, A. D., and Scammon, R. E., Relations between surface area, weight and length of the human body in prenatal life. *Proc. Soc. Exper. Biol. and Med.*, 1930, 27, 456.
 9. Schoenthal, L., Lurie, D., and Kelly, M., Urea clearance in normal and in dehydrated infants. Renal function in intestinal intoxication. *Am. J. Dis. Child.*, 1933, 45, 41.
 10. a. Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry, Vol. I. Interpretations. The Williams and Wilkins Co., Baltimore, 1931, pp. 345-347.
b. Smith, H. W., *The Physiology of the Kidney*. Oxford University Press, New York, 1937.
 11. a. McCance, R. A., and Young, W. F., The secretion of urine by newborn infants. *J. Physiol.*, 1941, 99, 265.
b. Young, W. F., Hallum, J. L., and McCance, R. A., The secretion of urine by premature infants. *Arch. Dis. Child.*, 1941, 16, 243.
 12. a. Cope, C. L., Studies of urea excretion. VIII. The effects on the urea clearance of changes in protein and salt contents of the diet. *J. Clin. Invest.*, 1933, 12, 567.
b. Goldring, W., Razinsky, L., Greenblatt, M., and Cohen, S., The influence of protein intake on the urea clearance in normal man. *J. Clin. Invest.*, 1934, 13, 743.
c. Farr, L. E., The effect of dietary protein on the urea clearance of children with nephrosis. *J. Clin. Invest.*, 1936, 15, 703.
 13. Shannon, J. A., Glomerular filtration and urea excretion in relation to urine flow in the dog. *Am. J. Physiol.*, 1936, 117, 206.
 14. Barnett, H. L., Renal physiology in infants and children: I. Method for estimation of glomerular filtration rate. *Proc. Soc. Exper. Biol. and Med.*, 1940, 44, 654.
 15. Herrin, R. C., Factors affecting the tests of kidney function. *Physiol. Rev.*, 1941, 21, 529.
 16. a. Peter, K., Untersuchungen über Bau und Entwicklung der Niere. Vol. I and II. G. Fischer, Jena, 1909-1927.
b. Clara, M., Vergleichende Histobiologie des Nierenglomerulus und der Lungenalveole. *Ztschr. f. mikr.-anat. Forsch.*, 1936, 40, 147.
c. Gruenwald, P., and Popper, H., The histogenesis and physiology of the renal glomerulus in early postnatal life: histological examinations. *J. Urol.*, 1940, 43, 452.
 17. a. Yllpö, A., Neugeborenen: Hunger; und Intoxicationssacidosis in ihren Beziehungen zueinander. *Ztschr. f. Kinderh.*, 1916, 14, 268.
b. Hoag, L. A., and Kiser, W. H., Jr., Acid-base equilibrium of newborn infants. I. Normal standards. *Am. J. Dis. Child.*, 1931, 41, 1054.
c. Marples, E., and Lippard, V. W., Acid-base balance of new-born infants. II. Consideration of the low alkaline reserve of normal new-born infants. *Am. J. Dis. Child.*, 1932, 44, 31.
d. Adolph, E. F., Postnatal development of water diuresis. *Am. J. Physiol.*, 1941, 133, 191.
e. Branning, W. S., The acid-base balance of premature infants. *J. Clin. Invest.*, 1942, 21, 101.
f. Rähkä, C. E., Säuglingsmortalität und Frühgeburtlichkeit—Über einige Neugeborenenprobleme. *Acta Paediatrica*, 1941, 28, 390.

INTUBATION STUDIES OF THE HUMAN SMALL INTESTINE. XXII. AN IMPROVED TECHNIC FOR THE STUDY OF ABSORPTION; ITS APPLICATION TO ASCORBIC ACID

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INTRODUCTION

Recent advances in the study of human nutrition indicate that a clinical deficiency of vitamins or other food factors may occur not only because of an inadequate intake or because of faults in storage or in utilization within the body, but also because of faulty absorption from the digestive tract. Consequently an accurate, direct and quantitative method of estimating the absorption of such substances in the human, under controlled conditions, is urgently needed. Various technics, including some developed in this clinic since 1934, have been used as a basis for the method which we will now describe and which seems to have certain advantages over those previously employed. At the same time, to illustrate the practical value of the method, we will present certain observations on its application to the absorption of ascorbic acid from the duodenojejunal region of normal subjects. The results have been of such a nature as to suggest that the method is trustworthy and applicable to the testing of other substances in health and in disease.

PREVIOUS METHODS

The tests of absorption utilized in the past have been inadequate for several reasons. In the procedures dependent on the concentration of the substance in the blood, such as Althausen's (1) galactose method, the rate of withdrawal of the test material from the blood for utilization, storage, and excretion cannot be controlled. Balance studies of total intake against total output in the feces fail to yield exact data on absorption because of the uncertain influence of bacterial action on the test substance in the bowel and of the possibility of its absorption and subsequent excretion into that organ. The employment of intestinal intubation, as originally suggested by Miller and Abbott (2), gives more direct data on absorption, though such specific technics as have been described (3 to 6) do not permit the intestine to function

normally or eliminate the possibility of certain quantitative errors. The closed-loop intubation method (3, 4) prevents the admixture of gastric, intestinal, hepatic, and pancreatic secretions from above, which presumably is necessary for normal absorption. The open-loop technic of Groen (5), because of the amount of the substance introduced at one time, distorts the ratio of volume to area, thereby interfering with the normal absorptive mechanism. In these methods, also, the damming back of large volumes of the test solution above the distal delimiting balloon often leads to an escape of some of the material beyond the test area, particularly in the presence of abnormal peristalsis associated with a diseased state.

CRITERIA OF A SATISFACTORY METHOD

To determine the absorptive power of the intestine for a given substance, a solution of the latter should be brought into contact with the absorbing surface under conditions that duplicate insofar as possible those that occur during the normal digestion of food: the motor and secretory functions of the intestine should not be disturbed and the concentration of the substance in solution should be within the range of normal for that section of the bowel. In order to insure such a physiological state of the intestine, care should be taken to avoid any mechanical obstruction, such as a balloon above the area under study, which might interfere with the normal progress of peristalsis or a free flow downstream of the secretions from the stomach, liver and pancreas. At the same time a method of obturation of the distal end of the intestinal segment should be chosen that will confine the test solution to the area under study and yet not initiate any unusual intestinal reaction. The chemical and physical characteristics of the material to be introduced, its volume and its rate of flow should be within the range of normal for the human as determined by such studies as those of Karr and Abbott (7).

A satisfactory absorption technic should not only operate within these physiological limitations, but also should be a quantitative procedure. This requires the maximal application of the test substance to a known area of absorbing surface. Since the concentration of the test solution falls at a rate proportional to the rapidity of absorption, such maximal application demands that a shorter loop be used for studying the more rapidly absorbed substances. The absorptive period, furthermore, should have a sharp starting-point, and also some means should be provided for terminating the procedure abruptly

¹ Sponsored by the Smith, Kline and French Laboratories, Inc.

at a fixed time and for withdrawing at that time all of the substance remaining within the test area.

TECHNIC AND CHEMICAL METHODS

The principle involved is the maintenance of a continuous perfusion from above downward of a physiologically acceptable solution through the lumen of a selected segment of small intestine, the functions of which are not disturbed by the procedure itself. Any portion of the bowel may be tested, but in our experiments the solution was introduced through one lumen of a double-lumened tube into the mid-duodenum (Figure 1, A'). Its rate of flow was 10 cc. per minute, which conforms to observations made in this clinic on the rate of gastric emptying. A concentration was chosen that would not produce intestinal irritation and yet would approach the normal absorptive capacity. From a point either 45 or 90 cm. downstream (Figure 1, B') the gut contents were withdrawn

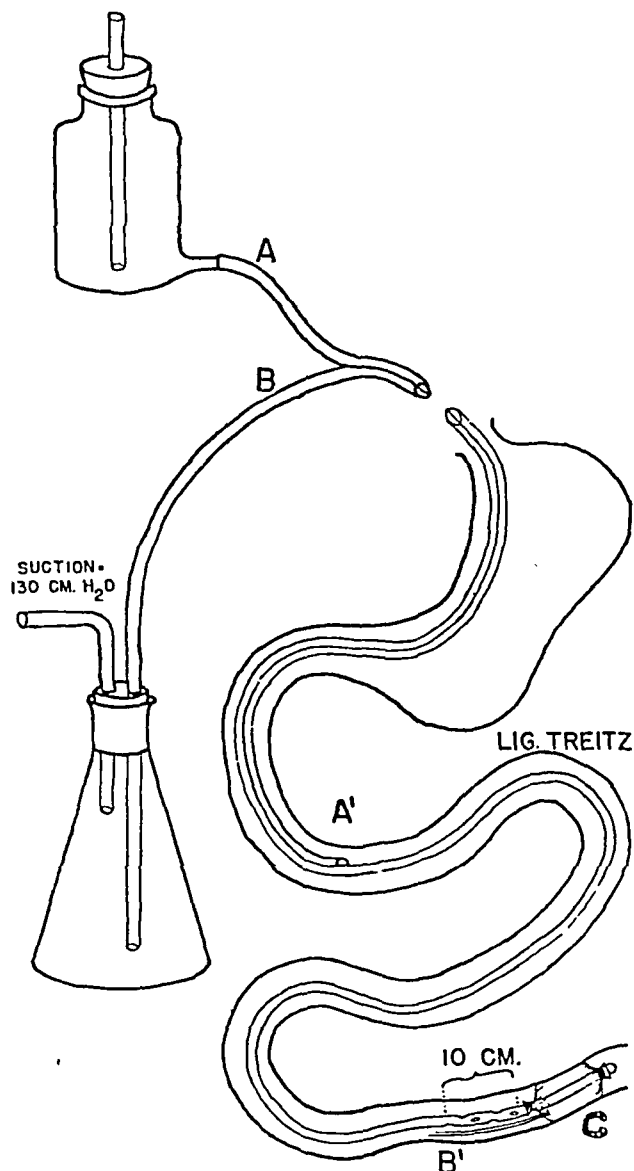


FIG. 1. DIAGRAM TO ILLUSTRATE THE TECHNIC EMPLOYED

through a suitable prolongation of the other lumen of the double-lumened tube by continuous suction as fast as they accumulated. Since the withdrawal capacity of the apparatus was 175 cc. per minute, greatly in excess of the possible rate of accumulation, a balloon at the distal end of the tube (Figure 1, C) assisted in diverting the flow but did not constitute an obstruction in the sense of producing a pooling of intestinal contents.

The apparatus consists of a 90 cm. double-lumened rubber tube, size 16 F, which has suitable fittings at the upper end for connecting the larger lumen B to a collecting flask and the smaller lumen A to a graduated Mariotte bottle. The lower end of the larger lumen is attached to either a 55 or a 100 cm. single lumen duodenal tube, size 12 F, which has multiple suction holes in the distal 10 cm. A rubber balloon C is tied to the end of the single tube but does not communicate with its lumen. The inflation of this balloon is controlled by a separate 1.5 mm. rubber tube which lies beside the larger tubing but communicates only with the balloon C. The intubation is performed in such fashion as to bring the distal end of the double lumened tube within the transverse portion of the duodenum. Thus a solution flowing through lumen A enters the mid-duodenum at A' while suction applied to lumen B constantly empties the intestine of its contents as fast as they arrive, either 45 or 90 cm. lower down at B'.

An experiment began with a flow of the solution into the duodenum (A') and the simultaneous withdrawal of whatever material was present at the distal end of the test area (B'). An interval of several minutes intervened before the return flow began. The experiment was abruptly terminated at the end of one hour by a cessation of the perfusion and the immediate introduction of 30 cc. of a 30 per cent solution of magnesium sulfate into the upper end of the test area (A'). This cathartic served to wash down all the contents and itself appeared in the collecting bottle in from 1 to 4 minutes, as indicated by the presence of carbon particles that had been injected with it. Tap water at a rate of 10 cc. per minute for one-half hour was then instilled at A', immediately following the magnesium sulfate injection, and the resulting flow of fluid aspirated at B' was collected in 10-minute fractions. The results obtained by the use of the 90 cm. technic in measuring absorption from a 10 per cent glucose solution over a period of 90 minutes are given as a working illustration in Table I. The presence of only a trace of glucose in the second 10-minute wash after the magnesium sulfate injection reveals the completeness with which the unabsorbed material may be removed.

For the determination of ascorbic acid in the blood, oxalated specimens were mixed, centrifuged, and samples withdrawn for analysis within a half hour after collection. The amount in the plasma was determined by the titration procedure of Farmer and Abt (8, 9) and by the photometric method of Mindlin and Butler (10). For the ascorbic acid content of the urine and intestinal specimens, samples were collected in flasks containing 10 cc. of glacial acetic acid and then refrigerated until the

TABLE I

The efficiency of the method as applied to the absorption of a 10 per cent solution of glucose from a 90 cm. segment of duodenum and jejunum

Time	Instilled at A'		Withdrawn at B'	
	Volume	Glucose	Volume	Glucose
<i>minutes</i>	<i>cc.</i>	<i>grams</i>	<i>cc.</i>	<i>grams</i>
0-30	300	30	265	13.8
30-60	300	30	310	16.7
60-90	300	30	320	19.8
90-93	30	} 0	225	7.0
	(30 per cent MgSO ₄)			
93-103	100			
103-113	100	0	60	trace
113-123	100	0	87	0.0
125-127	during removal of tube *		38	trace

Total gms. glucose injected	90.00
Total gms. glucose recovered	57.35
Total gms. glucose absorbed	32.65

* Represents results of continuous aspiration while withdrawing tube after completion of experiment.

experiment was completed. Then the volume was measured and suitable aliquots were removed either for the titration procedure or for dilution with 5 per cent acetic acid for the photometric method.

RESULTS WITH ASCORBIC ACID

The absorption of ascorbic acid in one hour from a 600 cc. solution containing 600 mgm. of this vitamin was determined by this technic in 19 experiments on 4 normal human adult subjects. To evaluate the effect of the previous ingestion of ascorbic acid on its absorption, experiments were performed on fasting individuals previously "saturated" by the daily intake, in addition to a regular diet, of 500 mgm. of ascorbic acid for one week as well as on "unsaturated" individuals. In 13 experiments, the absorption was measured for a segment extending 90 cm. beyond the instillation point in the duodenum. In the remaining experiments, the test segment extended only 45 cm. beyond the point of instillation. Because a water solution of ascorbic acid in this concentration may be sufficiently hypotonic to be irritating, as shown in dogs by Dennis (11), physiological saline was in some instances used as a solvent.

In Table II are given the results of 5 groups of experiments performed under the various conditions just stated. The amount of ascorbic acid absorbed by "saturated" and "unsaturated" subjects was approximately the same when either a

TABLE II

The absorption of ascorbic acid from the duodenum and upper jejunum in the normal human adult as determined by the described technic

Subject	Status of ascorbic acid nutrition	Ascorbic acid in fasting blood plasma	Length of per-fused segment	Nature of solution	Ascorbic acid absorbed in 1 hour	
					Total	Per sq. m.
		mgm. per cent	cm.	600 mgm. in 600 cc.	mgm.	mgm.
Br	unsaturated	0.9	90	watery	261	161
Hn	unsaturated		90	watery	369	187
Hn	unsaturated		90	watery	330	167
Cs	unsaturated		90	watery	374	174
Br	saturated	1.7	90	watery	349	215
Br	saturated	1.3	90	watery	325	201
Cs	saturated	2.0	90	watery	280	132
Cs	saturated	1.3	90	watery	252	119
Br	saturated	1.0	90	saline	354	219
Br	saturated	1.3	90	saline	232	143
Hn	saturated	1.6	90	saline	253	128
Cs	saturated	1.6	90	saline	253	119
Bt	saturated	1.4	90	saline	188	132
Br	unsaturated	0.4	45	saline	292	179
Hn	unsaturated	0.9	45	saline	290	147
Bt	unsaturated	0.7	45	saline	197	139
Br	saturated	1.2	45	saline	283	175
Hn	saturated	1.1	45	saline	308	156
Bt	saturated	1.1	45	saline	366	258

watery or a saline solution was used. Likewise the tonicity of the test solution did not alter the rate of absorption in the saturated individuals. Furthermore, the absorption of this vitamin from a segment terminated at 45 cm. beyond the point of instillation was of the same degree as that in a segment terminated at 90 cm. Although the absorption of ascorbic acid varied over a wide range, i.e., 188 to 374 mgm., or 31 to 62 per cent of the total injected, every subject absorbed an amount in one hour far in excess of the optimal daily intake of 100 mgm. as determined by Ralli *et al.* (12). When calculated on the basis of surface area, the amounts absorbed varied over an equally wide range.

DISCUSSION

Our experience with this method emphasizes its inherent advantages. Direct duodenal instillation avoids the variability of gastric emptying and allows the gut to fill and empty from above downward. Absorption, therefore, takes place without the participation of abnormal peristalsis, such as is

SPECIFIC ANTIPNEUMOCOCCAL IMMUNITY IN RELATION TO THE CHEMOTHERAPY OF PNEUMONIA^{1,2}

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The findings recorded in this report are concerned with the effect of chemotherapy on the occurrence of type specific pneumococcal antibodies in patients suffering from pneumonia. The incidence of the development of demonstrable specific immunity has been determined with samples of serum obtained at frequent intervals from patients with pneumonia, who were treated with either sulfapyridine or sulfathiazole. In addition to the quantitative aspects of the immunological information derived from the observations, the serological results have also formed the basis for an analysis of the significance of the presence or absence of measurable specific antibodies, in relation to the outcome of the infection in patients treated with either one drug or the other.

The patients available for the immunological study presented varied clinical courses and suffered from pneumonia of varying degrees of severity. Approximately one-third of the group had bacteremia. Although the majority of them recovered promptly from the infection, some had complicating febrile episodes, with and without bacteremia, and still others died. By correlating the different kinds of clinical courses with the results of the serological tests for specific antibodies, the attempt has been made to reconstruct the course of the disease on the basis of the immunological findings. In elaborating the second phase of the problem, the study has also included clinical and postmortem findings of other patients with pneumonia who were not used for serological study, but who were serviceable in the assay of factors that appeared either to promote or retard recovery, or were associated with fatal termination.

¹ This study was supported by a grant donated by Mr. Bernard Baruch.

² Part of the results contained in this article was presented at the annual meeting of the Association of American Physicians, Atlantic City, N. J., May, 1941.

Although most of the patients were treated with drugs alone, in a few instances, which will be subsequently noted, therapeutic antipneumococcal serum was also administered.

Wood and Long (1) made observations on the development of type specific antibodies in 12 patients with pneumonia who were treated with sulfapyridine. The antibody response was found to be similar to that of untreated cases of pneumonia. Finland, Spring and Lowell (2) made serological studies on more than 100 pneumonic patients treated with drugs and concluded that the antibody response was comparable to that resulting from spontaneous recovery. They found that protective antibodies rarely developed before the sixth day of disease. Kneeland and Mulliken (3, 4) have reported the results which they obtained with type specific precipitin reactions performed with specimens of serum from patients with pneumonia. Kneeland and Mulliken obtained specific precipitin reactions in only 4 cases out of 19 treated with sulfapyridine, whereas, again using the precipitin reaction, they found positive reactions in 16 of 21 patients who had been treated with sulfathiazole.

In experimental studies, Whitby (5), and McIntosh and Whitby (6) observed that mice cured of pneumococcus infections with sulfapyridine, subsequently exhibited active specific immunity against the type of organism used in the therapeutic tests. The latter authors concluded that sulfapyridine in no way altered the development of an immune response. MacLeod (7) described results which emphasized the close parallelism between the dosage of pneumococci amenable to the curative action of sulfapyridine and the dosage of heat killed organisms which induced active immunity. He further demonstrated that when sulfapyridine and antipneumococcus serum were employed simultaneously, recovery of mice could be obtained with combined doses in amounts that individually were ineffective.

Plummer, Liebmann, Solomon, Kammerer, Kalkstein, and Ensworth (8) have reported the results obtained from the comparative treatment of 607 patients with pneumonia. The difference in the fatality rate between the patients receiving drug plus serum as compared with those receiving drug alone was negligible. Dowling, Abernethy, and Hartman (9) have advocated the use of combined treatment in patients past 40 years of age. Bullowa, Osgood, Bukantz, and Brownlee (10) obtained a lower death rate with sulfapyridine alone than with any

other method. Bukantz, Bullowa, and deGara (11) suggested that determinations of pneumococcal polysaccharide in the blood of patients with pneumonia might serve as an indication for the use of serum.

MATERIALS AND METHODS

Patients. The cases of pneumonia were selected from those admitted to the Wards of the Third Medical Division of Bellevue Hospital during the fall, winter, and spring of 1939-40 and of 1940-41. Serological tests were limited to infections due to pneumococcus Types I, II, III, V, VII, and VIII.

Serum. Repeated bleedings, usually every 2 to 3 days, were obtained from the patients during their stay in the hospital. The first sample was taken before treatment was begun. Serum, separated in the usual manner with sterile precautions, was regularly employed.

Cultures. Stock cultures of the appropriate pneumococcal types were maintained at maximum degrees of virulence for mice. Culture dilution of 10^{-7} was regularly fatal. Sixteen to 18 hour blood-broth cultures were used for injection.

Tests. Passive protection of mice. Two-tenths cc. of serum diluted to 0.5 cc., by adding 0.3 cc. of physiological salt solution, was regularly employed for injection. Ten-fold serial dilutions of the broth cultures of type specific pneumococci were made in sterile broth, so that 0.5 cc. of each dilution equaled 0.1 cc. to 0.000001 cc. of original cultures. The 0.5 cc. of diluted serum and the 0.5 cc. of dilutions of culture were drawn into the same syringe and injected intraperitoneally into mice. Mice receiving only cultures in dilutions of 10^{-5} and 10^{-6} were used as virulence controls in each experiment. Routinely, the protective power of sera was tested against 4 dilutions of culture ranging from 10^{-3} to 10^{-7} . In some instances, when mice survived 10^{-3} , the titration was carried to 10^{-1} . All mice were observed for 7 days.

In order to exclude the remote possibility that the sulfapyridine or sulfathiazole contained in samples of serum obtained during the period of chemotherapy accounted for the protection of mice, many specimens of sera were tested for curative effect against pneumococci heterologous in type to that infecting the patient. In no instance did heterologous protection occur.

Agglutination. Although tests for the presence of type specific agglutinins were not made with all samples of sera, a sufficient number of titrations were performed to indicate the results in comparison with the protection tests. Five-tenths cc. of broth culture plus 0.5 cc. of undiluted serum constituted a final serum dilution of 1 to 2. Other serum dilutions of 1 to 5, 1 to 10, 1 to 20, and 1 to 40 were also employed. Final readings were made macroscopically after the tubes had been incubated in the water bath at 37.5° C. for 2 hours and allowed to stand in the icebox overnight. Type specific agglutination was deemed to be present only when a disc or coarse granulation, characteristic of type specific pneumococcal antigen-antibody reactions, developed.

Two hundred and seventy-five patients have been treated during the 2 year period of study. Twenty-five have died (9.0 per cent). Six additional patients, admitted in a moribund state, died within 6 to 20 hours of admission. Cases with pneumonitis which clearly terminated some other fatal disease have not been included. Cases of acute lower respiratory infection, which were usually mild and of undetermined etiology, have also been excluded.

Ninety-four patients were used in the serological studies. Thirty-one had bacteremia. Fourteen were considered to have protracted febrile illnesses of clinical significance. Ten of the patients died.

The results of the serological tests are summarized in Table I. The presentation of data is so arranged that results referable to the frequency of development of type specific antibodies, and to the approximate time in the course of the disease at which they became demonstrable, are indicated in two separate columns under "Type specific antibodies." Additional columns of tabulation indicate the time of the first positive test in relation to the duration of chemotherapy. The latter arrangement has been made in order to estimate the significance of demonstrable excess antibodies with respect to the outcome of the infection. The bearing of these findings on problems relating to clinical events and the immunological states of the patients will be subsequently discussed.

Frequency of development of type specific antibodies. From the data in Table I, it may be seen that 65 (69 per cent) of the 94 patients developed specific antibodies, the excess of which was sufficiently great to confer passive protection on mice. Among the 84 patients who recovered, 59 (70 per cent) possessed demonstrable serological immunity; among 10 patients who died, 6 (60 per cent) gave positive tests.

Separating the cases of Type III pneumococcus infection, there were, among the other types, 58 (80 per cent) instances of the appearance of antibodies among 72 cases. Of the cases of Type III pneumococcus pneumonia, only 8 (36 per cent) out of 22 produced effective passive protection. Serological studies in Type III pneumococcus pneumonia, as well as experimental observations, have uniformly indicated the poor specific antigenicity of Type III pneumococci. The findings in patients receiving chemotherapy are in accord

with the other studies concerning the formation of antibodies evoked by Type III pneumococci.

It may also be noted from Table I that among the 31 bacteremic patients, 16 (51 per cent) possessed demonstrable excess antibodies, whereas of the 63 non-bacteremic patients, 49 (77 per cent)

were found to develop measurable type specific immunity. Whether or not the greater amount of antigen present in association with the bacteremia combined with antibody so that the excess was reduced to an unmeasurable minimum, or whether the antibody production was less under the condi-

TABLE I

Occurrence of type specific antibodies in patients with pneumonia treated with sulfapyridine or sulfathiazole

Pneumo- coccus types	Number of cases		Bacteremia		Type specific antibodies		Appearance of antibodies in relation to duration of therapy				
	Recovered	Died	+	—	Passive protection +	Day of disease 1st positive test	Before	To 3rd hospital day †	"Late"	None	
I	21	0	5		3	6th to 12th av.: 9th		1	2	2	
				16	15		2	5	8	1	
II	28	3*	11		4	5th to 13th av.: 8th	1		3	7	
				17	15		3	4	8	2	
			3		0					3*	
			0								
III	18	4	2		0	10th to 12th 7th: 1 case					
				16	6			2	4	10	
			1	1	<i>I</i> †						
			3	2				2	<i>I</i>		
V	5	0	2		2	7th to 10th av.: 8th			2		
				3	3						
								1	2		
VII	9	3	3		3	5th to 9th 15th: 1 case		1	2		
				6	6		5	1			
			2	2	<i>I</i>		<i>I</i>				
			1	1	<i>I</i>						
VIII	3	0	2		1	13th			1	1	
				1	1			1			
TOTAL	84	10	31	63	65		15	18	32	29	

* Patients died in less than 24 hours after admission.

† Italics indicate fatal cases.

‡ Includes cases in which 1st test was negative, but passive protection obtained on 2nd or 3rd hospital day. "Late" signifies the presence of passive protective power after the 3rd hospital day.

tions of excess antigen, has not been determined. The difference in the frequency of excess antibodies between the bacteremic and non-bacteremic group appears to be definite.

Approximate time of appearance of antibodies. In view of the fact that samples of blood were taken every 2 to 3 days instead of daily, the results listed in Table I under "Day of disease of 1st positive test" represent the approximate time at which the immune response was first demonstrable. From averaging all of the data, the protective power of the sera was found to appear in the majority of instances on about the 7th to 10th day. In a few cases, positive tests were not apparent until the 12th to 13th day. An exception to the general results may be noted in the cases of Type III pneumococcus infection, and also in 2 of the 3 cases of pneumococcus Type VIII pneumonia, in which the development of sufficient antibody to afford passive protection to mice was regularly delayed until the 10th to 13th day.

Titre of specific antibodies. Each sample of serum was regularly tested in mice against 10^{-3} dilution of culture which corresponded to 10,000 minimal lethal doses of a virulent strain of the homologous type. With sera obtained from cases of infection due to Types I, II, V, and VII pneumococci, the titre of specific immunity was sufficiently high in the greatest number of instances to afford protection against the greatest dose used in the routine tests. Tests with greater amounts of culture were not done frequently enough to determine the maximum amount of protective antibodies which were present. However, the constancy of the results has indicated that the quantitative production of antibodies by patients was not significantly impaired by chemotherapy. In the patients with Type III pneumococcus pneumonia, as well as in the 3 patients with Type VIII pneumococcus pneumonia, the protective power of sera did not exceed 10^{-4} dilution of culture.

Type specific agglutination. Type specific agglutinins were found to be present in 44 (59 per cent) of the 74 sera that were tested. In a number of specimens, the protective power of serum was demonstrable but agglutination did not occur. The apparent difference in the results obtained with the 2 tests is dependent upon the fact that the passive protection of mice is a more delicate

method of determining the presence of type specific antipneumococcal antibodies than is the agglutination reaction. Consequently the presence of protective capacity in the absence of agglutination is an expected finding which is dependent upon the quantity of specific immune principles in the samples of sera.

Time of appearance of demonstrable antibodies during the course of chemotherapy. The data in the columns under the heading "Appearance of antibodies in relation to duration of therapy" are arranged for the purpose of analyzing the incidence of demonstrable type specific immunity in relation to the outcome of drug treatment. From the results recorded under the heading "Before," it may be noted that in 15 patients, the samples of serum obtained on the day of admission—before treatment was begun—contained specific protective antibodies. In 18 additional patients, specific antibodies had become demonstrable by the 3rd day after admission. This group has been separated because of the fact that the first 3 days most often constitute the "crucial" period of therapy. During this period, essential recovery or marked improvement may occur; a continued illness may persist; or the patient may become progressively worse. In analyzing the factors that influence the outcome of the infection, therefore, it is a matter of particular interest to evaluate the immunological status of the patients during the early phase of treatment. Combining the cases in which specific antibodies were present before treatment was instituted with the cases in which protective power became evident by the 3rd day of hospitalization, it may be noted that in 33 instances excess antibodies were present in the early phase of chemotherapy.

Under the column headed "Late," in Table I, there is a list of 32 cases in which measurable amounts of free circulating specific antibodies did not appear until after the 3rd hospital day. In some instances, chemotherapy was being continued when the protective action of the serum became positive, and, in other instances, sulfonamide treatment had ceased before specific immunity was demonstrable. Since, however, the extent of therapy was determined solely on the basis of the clinical condition of the patient, the serological findings did not influence the duration of sulfonamide administration. Consequently, in this

group, a relationship of the time of appearance of positive immunological findings to the response to chemotherapy was not clearly defined.

In the last column of Table I, there are listed 29 patients in whose sera type specific antibodies were not demonstrable at any time during the course of the illness. In connection with the implied immunological difference between the patients with and without demonstrable antibodies, it is important to re-emphasize the fact that the failure of a sample of serum from a patient to protect mice by passive immunization may not be final proof of the total absence of specific antibodies. It has been demonstrated experimentally that effective active immunity may be developed, even though the serum of the animal does not afford protection to other animals. Consequently the difference between the patients with protective antibodies and those without them may be quantitative rather than entirely qualitative.

In summarizing the findings that deal with the time of appearance of antibodies in relation to period of drug treatment, the results demonstrate that in 33 of the cases (35 per cent), specific immunity was present during the first 3 days of treatment, either at the time of admission of the patient (15 instances) or within the early period of hospitalization (18 instances). In 32 patients (34 per cent), excess antibodies became demonstrable at varying periods later than the 3rd day of hospitalization. In 29 patients (31 per cent), the various samples of sera, which were tested, failed in all instances to confer passive protection.

The fact that the cases which developed free circulating antibodies are scattered essentially evenly among the 3 groups of patients indicates the absence of definite correlation between the period of administration of sulfonamide drugs and the functioning of the mechanism of antibody production.

Although the selection of cases included in this study was not determined by the type of drug (sulfapyridine or sulfathiazole) used for treatment, an analysis of the 2 groups reveals the fact that 62 of the patients received sulfapyridine and 29 received sulfathiazole. The patients dying in less than 24 hours after admission are omitted. The results obtained following the 2 different medications are recorded in Table II according to pneumococcus types causing infection and the incidence

of immune response as demonstrated by the protective capacity of sera for mice.

TABLE II

Pneumo- coccus types	Num- ber of cases	Sulfapyridine		Num- ber of cases	Sulfathiazole	
		Specific antibodies (Passive protection)				
		Present	Absent		Present	Absent
I	16	12	4	5	5	0
II	16	12	4	12	8	4
III	13	6	7	9	3	6
V	4	4	0	1	1	0
VII	10	10	0	2	2	0
VIII	3	2	1	0	0	0
Total	62	46	16	29	19	10

From the tabulated data it is seen that of the 62 patients treated with sulfapyridine, 46 (74 per cent) developed demonstrable type specific antibodies, and that the sera from 19 (65 per cent) of the 29 patients receiving sulfathiazole afforded similar protection. In view of the relatively small number of patients included in each group, the difference in the results does not seem significant.

In considering the clinical courses of the patients who were used for serological observations, recovery was uneventful in 70. Fifty-five (75 per cent) developed type specific antibodies; in 15, none were detected. The nature of the recovery just mentioned indicates that there was no apparent difference in the progress of the patients possessing demonstrable antibodies and the others.

Of the 14 patients whose recovery was significantly delayed because of protracted febrile illness, in 5, type specific antibodies were demonstrable early after hospitalization, in 5 others, they appeared during the late period, and in 4, none were evident up to the time of discharge from the hospital. The majority of the patients who pursued stormy courses were among those with bacteremia. As stated earlier in the report, 16 (51 per cent) of the 31 patients with bacteremia developed measurable amounts of specific immunity. When the clinical records of the 16 with antibodies and the 15 without were compared, no clear-cut differentiation could be made. There were certain clinical impressions that in the bacteremic patients without demonstrable antibodies, recovery may in some instances have been retarded or

resolution may have been delayed to a greater degree than in bacteremic patients who developed specific antibodies. However, in view of the variables in the factors of pneumonia which may be referable, on the one hand, to intensity of infection or the nature of the complications, such as empyema or sterile pleural effusion, or, on the other, may depend upon the general physical status of the hosts of the infection, it has not been possible, among the bacteremic patients, to account for differences in clinical behaviour by an analysis of objective data obtained by the serological methods used in the present study.

Individual charts are presented which illustrate different types of courses in relation to the immunological finding.

Figure 1 is that of a patient who had, on admission, primary pneumonia and bacteremia due

to pneumococcus Type I. He came to the hospital on the 2nd day of the disease. Because of clinical evidence of rapid improvement, drug therapy was deliberately stopped, after 13 grams had been given in 30 hours, in order to observe the course in an early case receiving limited therapy. The subsequent rise in fever seemed to represent a relapse of pneumonia, although bacteremia did not return. Permanent cure, following the re-administration of sulfapyridine, appeared to coincide with the appearance of excess antibodies.

Figure 2 is that of a patient who had, on admission, primary pneumonia and bacteremia due to pneumococcus Type II. His progress following chemotherapy was one of continued daily improvement leading to uneventful recovery. No excess antibodies were found in any of the speci-

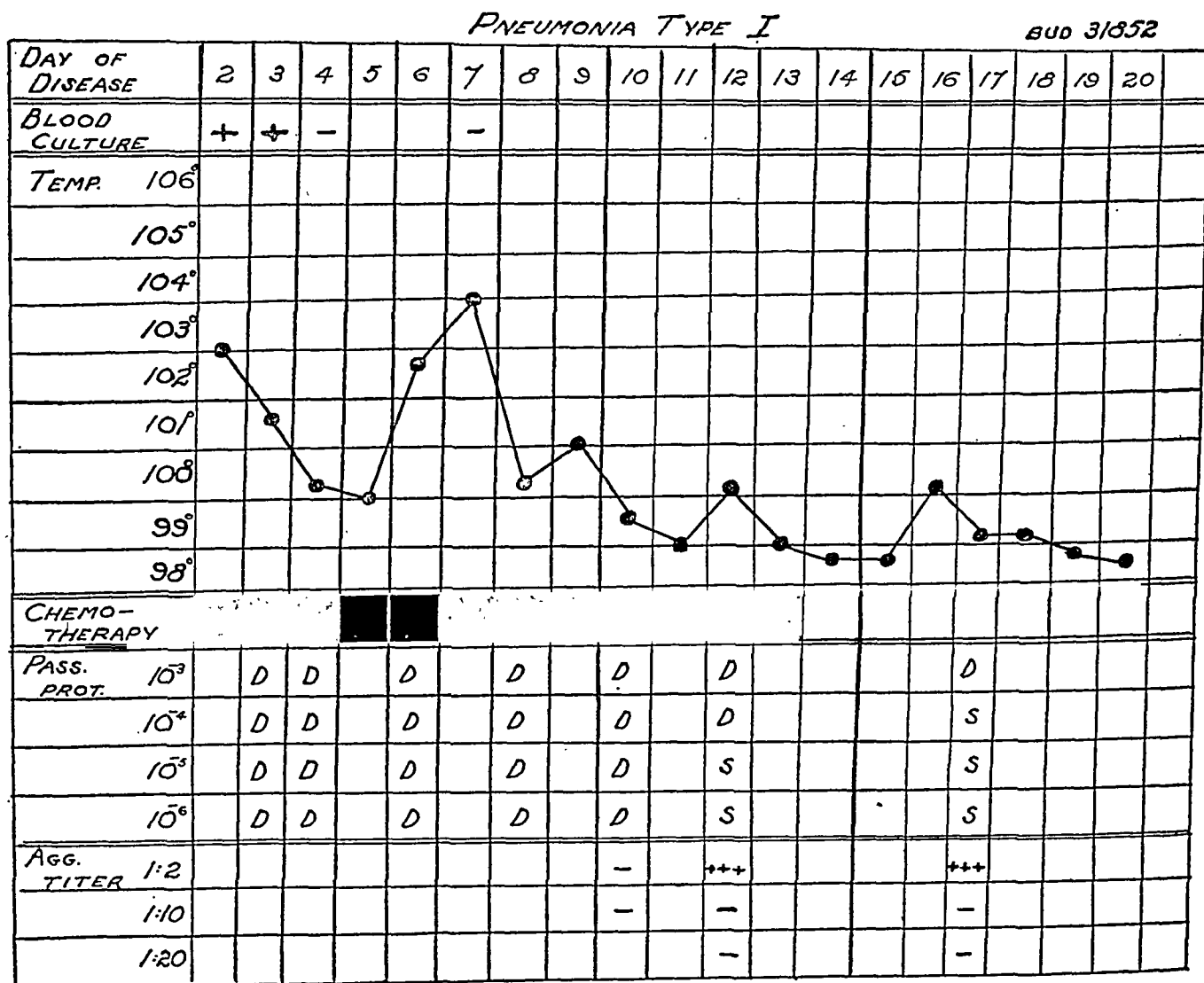


FIG. 1

PNEUMONIA TYPE II

AND 34570

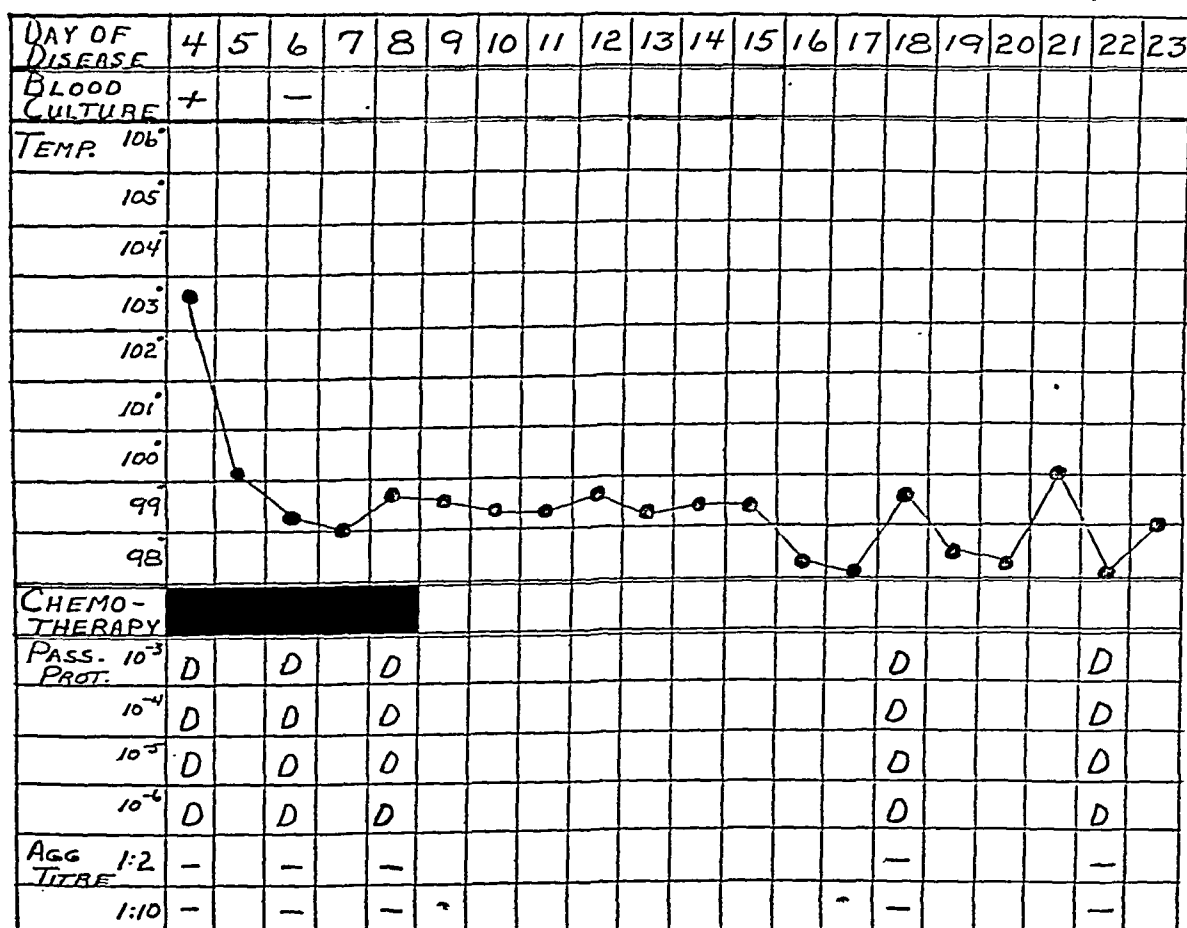


FIG. 2

mens of serum. The possible interpretations of the negative serological findings will be subsequently considered.

Figure 3 is that of a patient having pneumonia and bacteremia (pneumococcus Type VII), who had a protracted febrile illness which seemed clinically to be dependent, to a large degree, upon a recrudescence of active pneumonia and pleurisy, without empyema. It is unnecessary to detail the clinical findings save to indicate the fact that type specific antibodies were present before the secondary febrile episode began.

The chart of the 1st of the 3 bacteremic cases illustrates the clinical course in relation to the immunological mechanism that was frequently encountered. It seems likely that the febrile relapse could have been prevented either by continuing drug therapy longer or by introducing specific

antibodies at the beginning of therapy. However, in other patients the clinical-immunological course of events did not follow the same orderly procedure. The selected charts of the 2 other individual cases illustrate on the one hand, instances of progressive recovery following drug therapy without the mediation of demonstrable excess antibodies, and on the other hand, they exemplify conditions under which the presence of type specific antibodies did not in itself prevent certain febrile episodes that were interpreted clinically as being active pneumonic infection.

Fatal cases of pneumonia. The presentation of material has been arranged in order to particularize and to emphasize certain findings that were characteristically associated with death from pneumonia.

Age. Of the 31 fatal cases, including 6 which

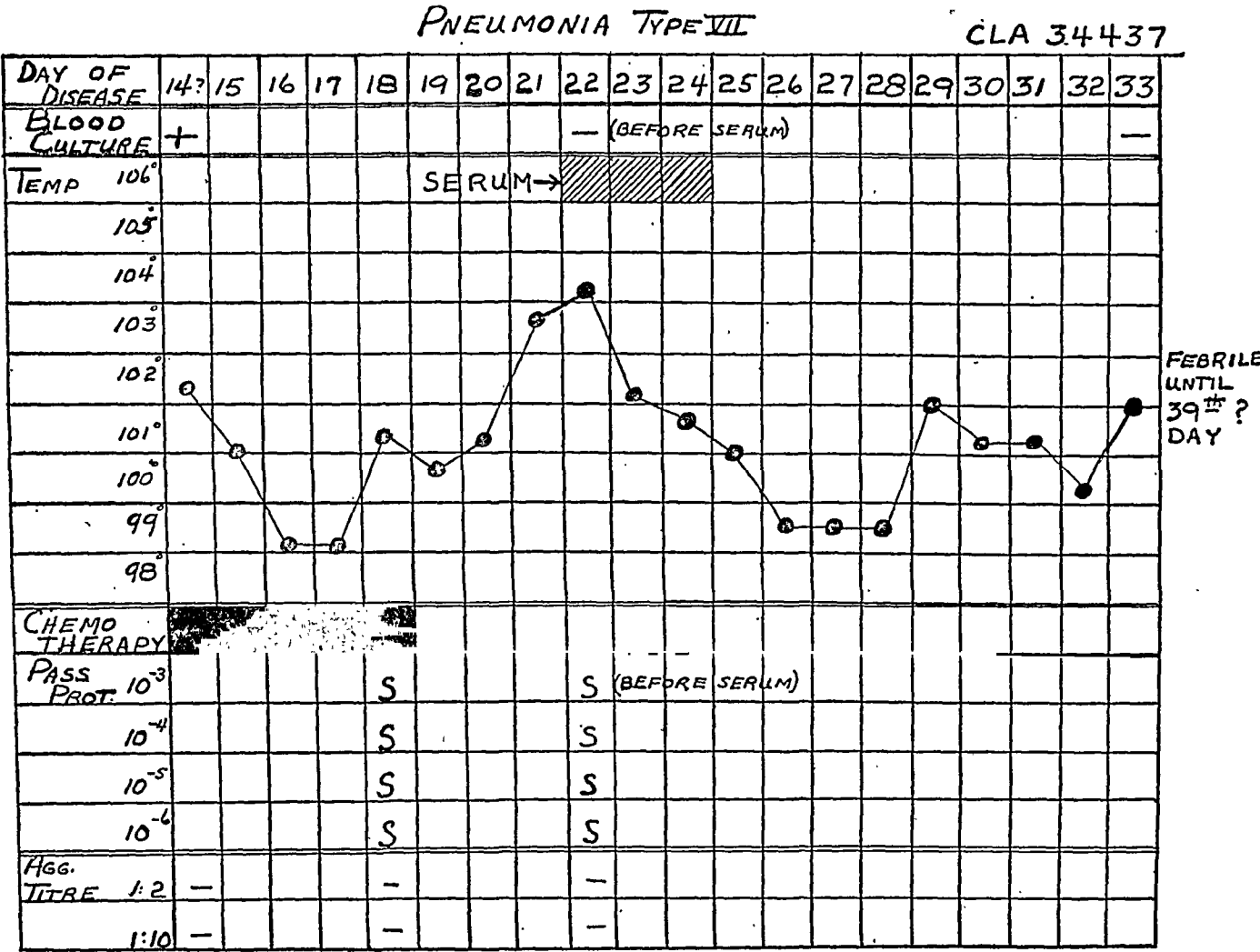


FIG. 3

were moribund on admission, the ages according to decades of life were: 1 between 20 and 30, 2 between 30 and 40, 3 between 40 and 50, 11 between 50 and 60, 10 between 60 and 70, 4 between 70 and 80. Eighty-four per cent were past 50 years of age.

Serological types of infecting pneumococci. The distribution of pneumococcal types was as follows: Type I, 2 cases; Type II, 8 cases; Type III, 8 cases; Type V, 2 cases; Type VII, 4 cases; Type XIV, 2 cases; and Types VI, XI, XII, XV, and XXV, 1 case each.

Bacteremia. In 21 of the 31 patients, pneumococci were recovered from the blood stream on the initial attempt; in 8, the first culture was sterile; in 2, no cultures were made. Seventy-two per cent had positive cultures on admission.

In 19 of the group, one or more subsequent cultures were taken. In 15, the bacteremia disappeared. Each of the 4 patients from whom subse-

quent cultures were persistently positive had endocarditis.³

Of the 12 cases in which a 2nd culture was not taken, 6 died on the 1st hospital day (all positive), and the remaining 4 died before the 4th day (3 positive, 1 negative); in 2, no cultures were taken at any time.

In addition to the statistical data just given, the fatal cases have been further analyzed in an attempt to identify significant factors that were associated with an unfavorable course. For this purpose

³ During the current winter, 2 patients with lobar pneumonia have been encountered in whom bacteremia persisted for as long as 6 days in spite of adequate chemotherapy. The administration of specific anti-pneumococcal serum therapy was followed in each instance by rapid improvement and prompt sterilization of the blood stream. One of the patients had Type I, and the other Type VIII pneumococcus pneumonia. Studies of the possible resistance of the strains to the effect of sulfonamides are now being made.

pose information derived from clinical and pathological sources has been correlated with bacteriological and immunological findings. Utilizing the cases concerning which the findings are complete, a majority may be divided into special groups, depending upon the conditions that appeared to be of special importance in association with unsuccessful treatment.

1. The severity of pneumonia at the time of hospitalization.
2. The presence of metastatic localized infections such as meningitis, endocarditis or pericarditis.
3. The coexistence of other diseases unrelated to pneumonia which were temporarily overshadowed by the manifestations of active infection.

1. Severity of primary pneumonia. On the basis of duration of life, 6 patients were admitted in a moribund state and died within 24 hours of hospitalization, either before treatment was begun or shortly thereafter. Two had meningitis, and 1 also had pericarditis, in addition to pneumonia.

Bacteremia was present in each of the 6 patients.

Immunological results. A single sample of serum was obtained from each of three of the patients. No passive protection was demonstrable.

The patients of this group comprised approximately 20 per cent of the total deaths.

TABLE III

Case	Age	Pneumococcus type	Blood cultures		Specific antibodies	Days in hospital
			1st	2nd		
L. K.	54	III	+	—	Serum * (2)	2
J. D.	56	VII	+	—	+	3
C. M.	55	III	+	—	+	3
R. K.	68	I	—	—	No test	2

* Numerals in parentheses indicate hospital day on which serum was taken with which positive protection tests were obtained.

A 2nd group of patients falling within this same category of severe primary pneumonia consisted of patients who lived until the 3rd hospital day, but in spite of therapeutic measures failed to survive. Selected data of bacteriological and immunological interest for 4 of the patients are given in Table III.

The course in this group of patients was characterized by a change of blood cultures from positive to negative and the appearance of type specific antibodies within a short period of time. In spite of the change in the bacteriological immunological balance following chemotherapy, the patients failed to survive. It seems not unlikely that although the extent of the infection, as evidenced by the blood culture, was decreasing during treatment, the initial severity was probably so advanced that even the loss of bacteremia and the acquisition of free circulating antibodies were ineffective in reversing the fatal course.

Six additional patients lived from 4 to 12 days. On clinical grounds, the diagnosis was limited to pneumonia. Their ages ranged from 50 to 74 years.

Bacteremia. In 4, blood cultures were negative throughout the period of hospitalization. In 2, the initial blood culture was positive but subsequent ones were sterile.

No serological tests were performed. No autopsies were obtained.

2. Metastatic pneumococcal complications. Meningitis, pericarditis, and endocarditis, singly or in combinations, were present in 10 (32 per cent) of the fatalities. Two died within 24 hours of admission. The remaining 8 survived 3 days or longer, and received intensive therapy.

Five cases had meningitis. Both drug and serum were administered to 2 of the 3 who lived long enough to receive continued treatment.

Three cases had pericarditis. One lived less than 1 day and another died on the 2nd day after admission. The 3rd patient, who lived 4 days, also had meningitis.

Four cases of endocarditis, which were proven by autopsy, and 1 unverified case, were observed among the 275 patients of the present series. It is interesting to note that in subsequent blood cultures, of 4 of the patients with endocarditis, bacteremia either returned after temporary absence or continued to be present for the 10 days' duration of the illness. Although the occurrence of persistent or recurring pneumococcal bacteremia among the cases of pneumonia observed during the 2 year period of this study was small, its association with endocarditis has been sufficiently striking to justify clinical suspicion of endocardial

involvement when bacteremia is refractory to continued treatment (see footnote number 3). No evidence has accrued which makes the present finding particularly unusual, even though endocarditis was proven in 1.4 per cent of the current series. It seems not unlikely that with the marked reduction in the total death rate due to pneumonia, the relative increase in pneumococcal endocarditis, which still remains uniformly fatal, accounts for the statistical difference. Of the 5 patients with endocarditis, 2 received serum. The course of the disease in the 5 patients was not different from that previously observed before the advent of chemotherapy.

Bacteremia was present in each of the 10 patients.

Immunological results. Of the cases with complications, no protective antibodies were demonstrated in single samples of serum from 3. In 4 others, 3 of which received serum therapy, free circulating antibodies were present in at least 1 test. In 3, no samples of serum were obtained.

3. *Coexistence of other diseases unrelated to pneumonia.* Seven patients, 22 per cent of the fatalities in the current study, comprised this group. Their illness was characterized by the fact that although lobar pneumonia was the most conspicuous part of the clinical diagnosis on admission, subsequent events, including findings at autopsy, proved that other diseases were of special importance in accounting for the fatal outcome. The patients of this group lived 5 days or longer. Cases in which pneumonitis was manifestly the terminal event in some other disease of maximum severity such as cerebral paralysis or uremia, have been excluded.

It is unnecessary to detail the individual clinical courses. Two of the patients had sudden terminal cardiac episodes during convalescence; in 2 senile female patients, gangrenous cystitis was found at autopsy; 1 patient with extensive anthrasilicosis of 5 lobes had a normal temperature for the last 6 days of life; 1 patient had an acute lead intoxication demonstrated at autopsy; 1 patient had an obscure and intractable encephalopathy. In each of the above instances, at autopsy, the pneumonia was resolving.

Bacteremia. Three of the patients had bacteremia on admission which disappeared within

48 hours after beginning chemotherapy. In the remaining 4, all blood cultures were sterile.

Immunological results. In 5 of the patients, repeated serological tests were performed, and in each instance, protection of mice was obtained with samples of serum obtained within the first few days after instituting chemotherapy.

In the foregoing analysis of the fatalities, by combining the cases that clearly belong in the 3 groups, but including only the moribund cases of the severe primary pneumonia group, it may be observed that approximately 74 per cent of the 31 deaths were identified according to special characteristics. In the selected groups, the fact that specific antibodies, when present, did not make up the deficiencies of chemotherapy appeared to be referable to the status of the patients' disease on admission. Of the remaining patients about whom information is not complete enough to warrant definite grouping, it is interesting to note the frequency with which bacteremia disappeared following chemotherapy.

DISCUSSION

The results described in this report indicate that the administration of sulfonamide drugs (sulfapyridine or sulfathiazole) did not significantly affect either the antigenic integrity of the infecting pneumococci or the immunological responsiveness of patients suffering from pneumonia.

With respect to the frequency of the development of demonstrable antibodies, the time of their appearance during the course of pneumonia, and approximate estimation of titre, the findings did not differ from the general experience derived from previous studies of untreated patients.

However, in spite of the absence of a direct effect of sulfonamide drugs on measured antibody response, indirect effects of chemotherapy on specific antipneumococcal processes were found to occur during the course of pneumonia and to be referable to quantitative alterations in antigen-antibody relationships. The studies contained in the present report serve as a basis for an analysis of some of the factors relating to the immunological course.

It has become a well established immunological principle that the antigen-antibody ratio in pneumococcus infections varies during the course of the

disease from excess antigen (pneumococci and soluble specific substance) and minimum antibody at the onset, to excess antibody and progressively decreasing antigen following recovery. Alterations in the two factors and their bearing upon the course of the disease are referable to changes in the extent of the infection during its progress in relation to antibody production. Specific anti-pneumococcal serum and the specifically acting sulfonamide drugs both alter the quantitative antigen-antibody relationships, but the result in each instance is obtained by different processes. Therapeutic antiserum operates through primarily increasing the antibody content and secondarily reducing the amount of antigen by opsonization

of pneumococci. The use of chemotherapy, on the other hand, is primarily successful by reducing the amount of antigen through the untoward effect of the drugs on the microorganisms. The decrease in antigen effected by the drugs brings about a relative increase in antibodies. It is also interesting to take into account the fact that specific antibodies are used up in the process of combining with pneumococci, whereas experimental studies have not up to the present time indicated that sulfonamide drugs are operative through permanent combination with bacterial cells. Consequently, increments of the drugs that are not inactivated or excreted may remain free for continuous action.

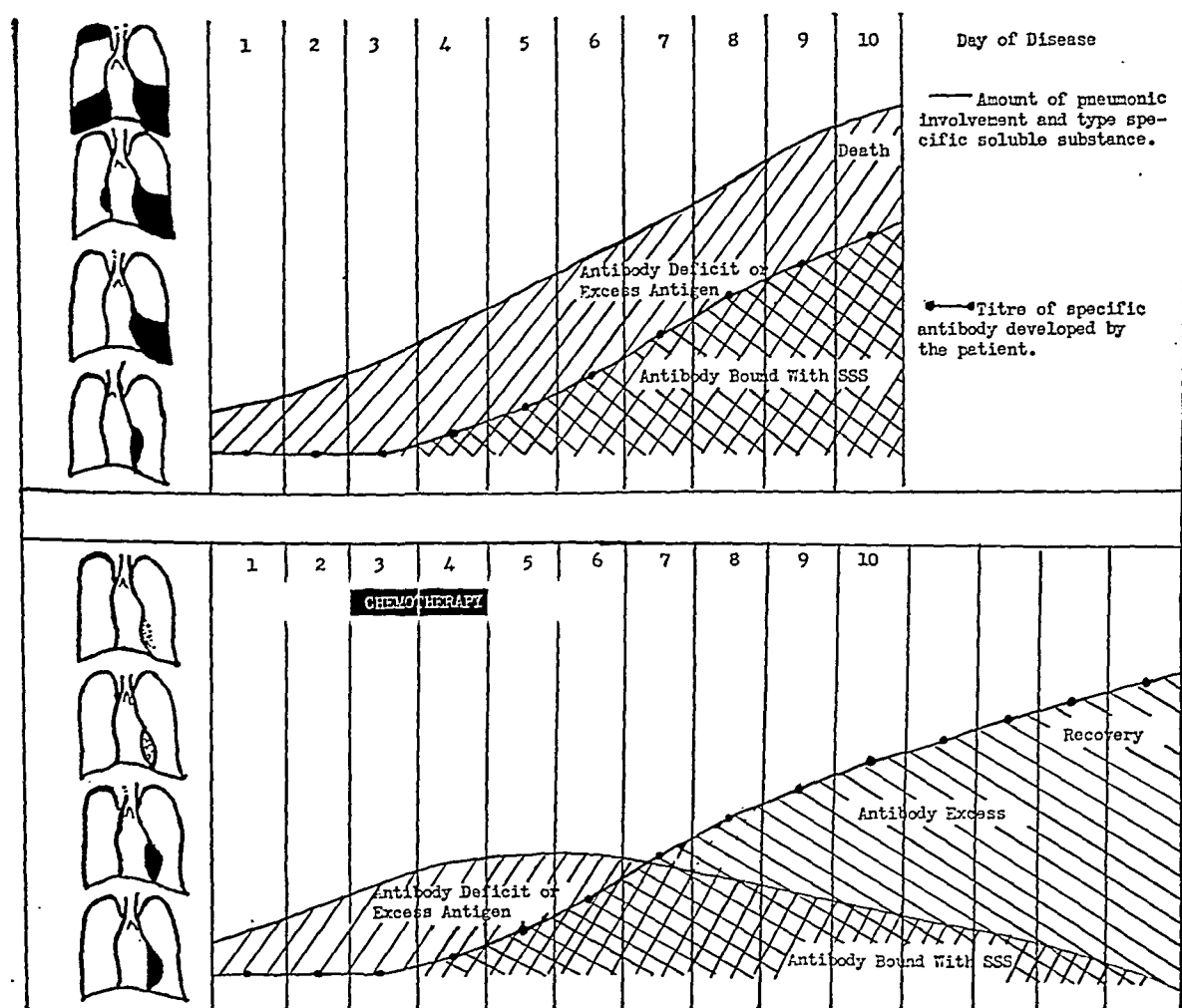


FIG. 4. THEORETICAL ANTIGEN-ANTIBODY RELATIONSHIPS DURING COURSE OF PNEUMONIA IN AN UNTREATED PATIENT AND IN 1 RECEIVING CHEMOTHERAPY

The effect of sulfonamide treatment on the antigen-antibody balance in pneumonia is illustrated graphically in Figure 4.

The data obtained from the present study frequently conformed to the course outlined in Figure 4. It appears not unlikely that this chemotherapeutic-immunological relationship occurs in the usual course of uncomplicated pneumonia proceeding to uneventful convalescence. However, with respect to ultimate recovery or death, the presence or absence of demonstrable specific antibodies did not uniformly separate the 2 groups. Exceptions were noted among both the patients who were successfully treated and also among the group who died. There were, for example, among the patients who recovered, a number of instances (24 listed in Table I) in which antibodies were not demonstrated during convalescence. Since, however, only free unbound antibodies are detectable by the methods employed, it is apparent that a negative serological reaction may be due to the fact that, (a) no antibodies have been developed following infection, or (b) all of the antibody was bound to antigen and, as a result, no excess was detectable. In the latter instance, the antibody production by the patient may have been highly effective in neutralizing antigen even though the serological tests were negative. In view of the limitations of the tests, it is not possible to conclude with certainty which of the interpretations is applicable to the findings in the recovered patients whose sera failed to confer passive protection.

In a consideration of the fatal cases, it is apparent that the extensiveness of the pneumococcal infection at the time of admission was frequently of primary importance in determining the outcome. For example, 20 per cent of the deaths occurred within 24 hours of admission. There were among the fatalities also an appreciable number who failed to recover even though specific antibodies were demonstrable in their serum. In correlating the clinical and pathological findings with the immunological results, significant factors associated with death were found to be of such a character that they were not decisively controlled by even the combined effect of chemotherapy and specific antibodies. Approximately 75 per cent of the deaths appeared to belong to the groups described in the body of this report.

Additional factors that may mitigate against satisfactory chemotherapy, such as intolerance of drugs by patients, or infections caused by drug-fast strains, or inactivation of sulfonamide compounds by para aminobenzoic acid or similarly acting substances, have not been included in the present study. The importance of their bearing upon optimum therapy with special reference to the use of specific serum therapy is apparent. However, that diverse conditioning elements, not regularly controlled by specific antibodies, not infrequently account for the unsuccessful use of chemotherapy, has been indicated in the present study.

SUMMARY

1. Ninety-four patients have been used for serological studies. Thirty-one had bacteremia. Seventy of the total number recovered from pneumonia uneventfully. Fourteen additional patients who also recovered had protracted febrile illness of clinical significance. Ten of the patients died.

During the 2 year period 1939-1941, among 275 patients with pneumonia, who, with a few exceptions, were treated with either sulfapyridine or sulfathiazole alone, 25 (9 per cent) died.

2. Sixty-three (69 per cent) of the 94 patients developed specific antibodies as determined by the passive protection of mice. Fifty-nine (70 per cent) of 84 patients who recovered possessed measurable specific immunity. Six (60 per cent) of 10 patients who died gave positive protection tests.

Eight (36 per cent) of 22 patients with Type III pneumococcus pneumonia produced detectable immunity. Fifty-eight (80 per cent) of 72 patients with Types I, II, V, VII, and VIII pneumococci gave positive serological tests.

3. In relation to day of treatment, rather than day of disease, excess antibodies were present in 15 instances *before* therapy was begun, in 18 instances by the 3rd day of hospitalization, in 32 instances after the 3rd day, and in 29 instances, all tests were negative.

4. A correlation between the clinical course of the disease and the immunological findings was made. Although the majority of the whole group of patients developed measurable amounts of specific immunity, the presence or absence of demonstrable antibodies did not, in some instances,

decisively separate the patients who satisfactorily responded to chemotherapy from others whose convalescence was delayed.

5. The fatal cases were divided into groups according to the factors that were found to be of special importance in determining the outcome.

6. The quantitative immunological relationships between infecting pneumococci and specific antibodies which occur during the course of pneumonia treated with sulfonamide drugs have been discussed.

BIBLIOGRAPHY

1. Wood, W. B., Jr., and Long, P. H., Observations upon the experimental and clinical use of sulfapyridine. III. The mechanism of recovery from pneumococcal pneumonia in patients treated with sulfapyridine. *Ann. Int. Med.*, 1939, 13, 612.
2. Finland, M., Spring, W. C., and Lowell, F. C., Immunological studies on patients with pneumococcal pneumonia treated with sulfapyridine. *J. Clin. Invest.*, 1940, 19, 179.
3. Kneeland, Y., Jr., and Mulliken, B., Antibody formation in cases of lobar pneumonia treated with sulfapyridine. *J. Clin. Invest.*, 1940, 19, 307.
4. Kneeland, Y., Jr., and Mulliken, B., Antibody formation in cases of lobar pneumonia treated with sulfathiazole. *J. Clin. Invest.*, 1940, 19, 735.
5. Whitby, L. E. H., Chemotherapy of pneumococcal and other infections with 2—(*p*-aminobenzenesulfonamido) pyridine. *Lancet*, 1938, 1, 1210.
6. McIntosh, J., and Whitby, L. E. H., Mode of action of drugs of the sulfonamide group. *Lancet*, 1939, 1, 431.
7. MacLeod, C. M., Chemotherapy of pneumococcal pneumonia. *J. A. M. A.*, 1939, 113, 1405.
8. Plummer, N., Liebmann, J., Solomon, J., Kammerer, W. H., Kalkstein, M., and Ensworth, H. K., Chemotherapy versus combined chemotherapy and serum in the treatment of pneumonia. A study of 607 alternated cases. *J. A. M. A.*, 1941, 116, 2366.
9. Dowling, H. F., Abernethy, T. J., and Hartman, C. R., Should serum be used in addition to sulfapyridine in the treatment of pneumococcal pneumonia? *J. A. M. A.*, 1940, 115, 2125.
10. Bullowa, J. G. M., Osgood, E. E., Bukantz, S. C., and Brownlee, I. E., The effect of sulfapyridine alone and with serum on pneumococcal pneumonia and on pneumococcus-infected marrow cultures. *Am. J. M. Sc.*, 1940, 199, 364.
11. Bukantz, S. C., Bullowa, J. G. M., and de Gara, P. F., Detection of free polysaccharide in the blood of pneumococcal pneumonia patients; prognosis and therapy. *Proc. Soc. Exper. Biol. and Med.*, 1939, 41, 250.

STUDIES ON BLOOD COAGULATION: A PROTEOLYTIC ENZYME PREPARED FROM CALCIUM AND PLATELET FREE NORMAL HUMAN BLOOD PLASMA¹

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The concept that blood plasma may contain a proteolytic enzyme system associated with blood coagulation is not new. Evidence has been accumulating from investigations in unrelated fields which points to the existence of such a system, but precise experimental evidence is lacking. The literature on fibrinolysis, considered as a problem related to blood coagulation, has been reviewed by Nolf (1), Hougardy (2) and Tagnon (3). Nolf reported observations on the fibrinolytic properties of serum obtained by shaking recalcified plasma with chloroform. He considers the enzyme responsible for this phenomenon to be an important factor in blood coagulation. Barker (4) in 1908 reported that fibrin contained a proteolytic enzyme system and explained his observations on the basis of entrapped mononuclear cells in the fibrin. Jobling and Petersen (5) published a series of articles in 1914 on the nature of serum antitrypsin. They found that when serum was treated with chloroform, ether, and other organic solvents, the antitrypsin was removed. They failed, however, to demonstrate any tryptic activity in the treated serum. Minot (6) investigated the clotting activity of chloroform on oxalated blood and reported that the chloroform removed antithrombin from the serum. Yamakawa (7) in 1918 studied the effect of ether, chloroform, and adsorption on serum and was able to obtain some proteolytic effects. He suggested that the effects of the reagents used depended upon the removal of an antitryptic substance.

¹ The expenses of this investigation were defrayed in part by a gift to Harvard University from the Smith, Kline and French Laboratories, Philadelphia, Pennsylvania; and in part by a grant given "in recognition of Dr. Francis W. Peabody's services to the Foundation" by the Ella Sachs Plotz Foundation.

² Graduate Fellow, Belgian American Educational Foundation, 1941-1942.

The subject of the effect of proteolytic enzyme systems, not of plasma origin, on blood plasma and fibrinogen has been reviewed by, and reinvestigated in, the work of Eagle (8) who showed that trypsin itself could cause blood coagulation. Eagle has suggested a coagulation reaction based on the Howell theory, in which calcium ion, blood platelets, or thromboplastin are replaced by trypsin. Ferguson and his coworkers (9, 10) have proposed a thromboplastic enzyme system, capable of breaking certain plasma complexes, which they consider related to blood coagulation. Until recently, however, the demonstration of the presence of a tryptic-like enzyme in plasma has been lacking.

Nolf (1) found that chloroform treated recalcified plasma possessed marked coagulation and fibrinolytic effect. Tagnon (3, 11), working exclusively on dog blood and using fibrinogen solution for testing purposes, repeated Nolf's observations and showed that when suitable concentrations of dog plasma preparations were used, the fibrin clot which first formed was redissolved. With still higher concentrations, no coagulation took place but fibrinogen was destroyed as evidenced by failure of the solution to clot when thrombin was subsequently added.

For purposes of purification, Tagnon precipitated diluted chloroform treated plasma at pH 6 and found that the precipitate contained essentially all of the activity of the preparation. He also showed that when the euglobulin fraction of this preparation was separated by dialysis, this fraction also contained most of the activity of the preparation.

Tagnon pointed out the similarity of action between his preparation and the action of trypsin on blood coagulation as described by Eagle (8). Apparently chloroform treated plasma was able, like trypsin, to produce thrombin from prothrom-

bin. Like trypsin also, was the failure of the chloroform treated plasma to coagulate fibrinogen unless prothrombin were present. The lytic effect of chloroform plasma on fibrinogen was also analagous to trypsin.

These observations have been confirmed in this laboratory. The present communication is an attempt to extend Tagnon's investigations to plasma of other species, to present further developments in preparation, and to present preliminary evidence that such preparations of plasma may constitute part of a plasma enzyme system. Investigations have shown that a non-cellular fraction of the plasma euglobulin has a rôle in blood coagulation independent of prothrombin and fibrinogen (12, 13). Therefore, the present observations also demonstrate the rôle which this "globulin substance" may play in a plasma enzyme system associated with blood coagulation. Lastly, the investigations attempt to show whether calcium ion or platelets are necessary in the elaboration of an active preparation. In the accompanying communication (14), the nature of the active agent will be discussed.

EXPERIMENTAL

Methods

Normal human blood was collected in sufficient 2.5 per cent sodium citrate to give a final concentration of 0.25 per cent of this salt.³ The plasma was obtained by centrifuging. Platelets and other formed elements were removed by recentrifuging the plasma at 4200 revolutions per minute for 30 minutes. To each 100 ml. of fresh cell free citrated plasma, 10 ml. of chloroform were added, without recalcification, and the mixture shaken vigorously for 1 minute in a tightly closed flask. It is important that the plasma be treated with chloroform within 12 hours of venepuncture. After some hours, a clot formed with considerable imbibition of the fluid portion of the mixture. The preparations were allowed to stand at room temperature, in the closed containers, until all of the clot had disappeared. This reaction for normal human plasma usually was complete in from 3 to 8 days. Rarely, a longer period than 8 days was required. When all the clot had disappeared, the flasks were opened and samples removed for bacteriological examination. The preparations were tested for prothrombin by recalcification or by Quick's procedure (15) after the addition of fibrinogen, and for fibrinogen by the addition of thrombin.

³ In many experiments, the citrate was replaced by oxalate (0.1 ml. of a saturated solution of potassium oxalate to each 10 ml. of blood) without altering the results in any way.

Both of these substances were absent. In no instance was the presence of bacteria, either by direct smear or by aerobic culture, demonstrated. The chloroform was removed by centrifuging and evaporation by a current of air. From this point on, any one of three methods of purification and concentration was employed. (1) The contents of the flasks were diluted with 10 volumes of distilled water, and the acidity brought to pH 6 by the addition of 1 per cent acetic acid. The supernatant liquid was syphoned off and the last portion of it removed by centrifuging. The precipitate was dissolved in 0.9 per cent sodium chloride to make a solution one-tenth the original volume of plasma. (2) Otherwise, the contents of the reaction flasks were transferred to cellophane tubes and dialyzed against running tap water at 8° C. for 3 or 4 days. The euglobulin precipitate so formed was separated from the supernatant liquid by centrifuging, and then dissolved in 0.9 per cent sodium chloride solution to make one-tenth the original volume of plasma. (3) To avoid dilution of the chloroform treated plasma and to shorten the time of dialysis, a third modification was occasionally employed, which was essentially a combination of the first two methods. The chloroform treated plasma was dialyzed for one and a half days and then, without dilution, the contents of the cellophane sacks were adjusted to pH 6. The resulting precipitate was again dissolved in an amount of 0.9 per cent sodium chloride solution equal to one-tenth the original volume of the plasma. This saline solution of the active material will be referred to as "chloroform plasma globulin solution." Modifications of the above standard procedures were sometimes employed for special purposes. These will be outlined at the appropriate time.

Fibrinogen was prepared by the method of Mellanby (16). Prothrombin was prepared by a modification of the method of Mellanby (16) in which diluted oxalated plasma was deprived of its fibrinogen by rapid heating to 56° C., cooling, and removal of the precipitate by filtration. The filtrate was brought to pH 5.3 by 1 per cent acetic acid, and the precipitate so formed dissolved in one-tenth the original plasma volume with 0.9 per cent sodium chloride solution. Both fibrinogen and prothrombin preparations were kept by rapid freezing to minus 45° C. and storing in a carbon dioxide refrigerator at that temperature. Thrombin⁴ was prepared from rabbit plasma by the method of Parfentjev (17, 18). Globulin substance was prepared either by acid precipitation (19) or by dialysis (2).

Titration of the activity of preparations and observations on fibrinolysis were made by placing 0.1 ml. of plasma or fibrinogen solution in 156 × 13 mm. test tubes, adding the requisite amount of the preparation to be studied and diluting the mixture to a total volume of 1.0 ml. with 0.95 per cent salt solution or imidazole buffer (20) at pH 7.4. All reagents and preparations used were adjusted to pH 7.4. All observations were made at 37.5° C. in a constant temperature water bath.

⁴ Supplied by Lederle Laboratories, Inc., Pearl River, New Jersey.

GENERAL PROPERTIES OF CHLOROFORM PLASMA
GLOBULIN SUBSTANCE

*Action of chloroform plasma globulin solution on
fibrinogen solution*

Tagnon showed that the addition of increasing amounts of his dog blood preparation to fibrinogen solution was not accompanied by clot formation. However, when a sufficient quantity was added, fibrinogen was completely destroyed, as indicated by the failure of thrombin to produce a fibrin clot. In a few preparations, we were able to obtain such a result. In most instances, however, coagulation of the fibrinogen occurred. This was considered to be due to small amounts of thrombic activity remaining in the preparation. It could be demonstrated that the lytic activity of the chloroform preparation was not due to its thrombin content, as shown below.

peated using pure thrombin solutions, clot formation occurred at all concentrations employed, but no lytic action was observed during a subsequent period of 24 hours. Seegers (21) likewise has failed to show any fibrinolytic property in his thrombin preparation.

*Action of chloroform plasma globulin solution on
normal plasma*

When the smallest quantity of chloroform plasma preparation which completely lysed fibrinogen, and which failed to clot fibrinogen solutions, was added to 0.1 ml. of plasma under standard conditions, a clot always occurred. Furthermore, much higher concentrations of the preparation could be added to plasma before clot formation was prevented. This is shown in the lower half of Table I. The sequence of events when

TABLE I
Activity of a chloroform globulin preparation on plasma and fibrinogen

Chloroform globulin preparation	Oxalated steer plasma	Oxalated fibrinogen	Saline	Clot formation	Dissolution	Thrombin solution after 30 minutes
ml.	ml.	ml.	ml.			
0.0		0.2	0.8	No clot 30 minutes	Dissolved 10½ minutes	Clot
0.1		0.2	0.7	Clot 2 minutes		No clot
0.3		0.2	0.5	No clot 30 minutes		No clot
0.8		0.2	0.0	No clot 30 minutes		No clot
0.0	0.1		0.9	No clot 30 minutes	Not dissolved 30 minutes Dissolved 12 minutes	Clot
0.1	0.1		0.8	Clot 1½ minutes		
0.3	0.1		0.6	Clot 1½ minutes		No clot
0.9	0.1		0.0	No clot 30 minutes		No clot

As shown in the upper part of Table I, when increasing amounts of the chloroform plasma globulin preparation were added to a fibrinogen solution, coagulation occurred only in the lower concentration. In all instances, a point was reached at which no coagulation occurred. Fibrinogen had been completely destroyed by the activity of the preparation. Furthermore, with increasing concentration of the chloroform plasma globulin, increasing lysis of the fibrin clot occurred. It could be concluded, therefore, that the preparation contained, in addition to thrombin, a powerful lytic material. When chloroform plasma globulin solutions were prepared according to the standard procedure in one-tenth the original volume of plasma, these results could always be duplicated. When these observations were re-

plasma was used instead of fibrinogen solutions is clearly indicated in Table I. With increasing concentration of the chloroform plasma preparation, there was coagulation followed by increased fibrinolysis until finally no coagulation occurred and no fibrinogen was present, as tested by the addition of thrombin. That the failure of the fibrinogen solution to clot after the addition of the chloroform preparation was not due to any antithrombic action of the preparation was demonstrated by the failure to obtain fibrinogen by chemical methods, when thrombin failed to coagulate the fibrinogen solution. The results would indicate that there was a marked inhibition of the lytic effect of chloroform plasma globulin preparation by plasma. What fraction of the plasma constituents is responsible for this inhibition is not

TABLE II

Action of prothrombin on the activity of a chloroform globulin preparation

Chloroform globulin preparation	Oxalated fibrinogen	Saline	Oxalated prothrombin	Clot formation	Dissolution	Thrombin solution after 30 minutes
ml.	ml.	ml.	ml.			
0.0	0.2	0.8	0	No clot 30 minutes	Dissolved 1½ minutes	Clot No clot No clot
0.1	0.2	0.7	0	Clot 2 minutes		
0.3	0.2	0.5	0	No clot 30 minutes		
0.3	0.2	0.4	0.03	Clot 1½ minutes	Dissolved 15 minutes Not dissolved 30 minutes Not dissolved 30 minutes	No clot
0.3	0.2	0.4	0.1	Clot 1½ minutes		
0.3	0.2	0.4	0.3	Clot 1½ minutes		

definitely established. It is known, however, that a plasma preparation containing a high concentration of prothrombin activity (17) will, as shown in Table II, reduce the lytic action of chloroform plasma to the same extent as plasma itself. This might suggest that prothrombin itself could be the inhibitor.

THE RELATIONSHIP OF CHLOROFORM PLASMA
GLOBULIN TO "GLOBULIN SUBSTANCE"
AND PLASMA EUGLOBULIN

Previous reports from this laboratory (12, 13) have shown the specific relationship of a globulin fraction of normal cell free plasma to the coagulation of blood. Tagnon has pointed out (11) that in the purification of his chloroform preparations

and dialysis (13) for the latter preparation. While it is known that both of these preparations have a marked effect in promoting the coagulation of hemophilic blood, they have no fibrinolytic power. Isotonic saline solutions of globulin substance or plasma euglobulin were treated with chloroform and permitted to stand at room temperature until the clot which had formed disappeared. The mixture was freed from chloroform and then was either precipitated at pH 6 or dialyzed against tap water at 10° C. in cellophane bags. In either case, the precipitate was dissolved in 0.9 per cent sodium chloride solution. Unlike the parent globulin substance or euglobulin, the preparation had marked fibrinolytic and fibrinogenolytic activity as shown in Table III. These results show that while globulin substance and plasma euglobu-

TABLE III

The activity of a preparation derived from chloroform treated globulin substance

Active preparation from globulin substance	Saline	Oxalated fibrinogen	Oxalated plasma	Clot formation	Dissolution	Thrombin added after 30 minutes
ml.	ml.	ml.	ml.			
0	0.9	0.1		No clot	Dissolved 10 minutes	Clot No clot No clot No clot
0.1	0.8	0.1		Clot 1 minute		
0.3	0.6	0.1		No clot		
0.5	0.4	0.1		No clot		
0	0.9		0.1	No clot	None 30 minutes Dissolved 20 minutes No clot	Clot No clot No clot No clot
0.1	0.8		0.1	Clot 1 minute		
0.5	0.4		0.1	Clot 1 minute		
0.8	0.1		0.1	Clot 1 minute		

similar methods were used to those employed in making globulin substance and plasma euglobulin preparation. He suggested that there might be a relationship between these two substances. Globulin substance and plasma euglobulin were prepared by acid precipitation (20) for the former

lin are essentially inactive as enzymes, they can readily be converted, without the addition of calcium, to proteolytically active substances by simple treatment with chloroform. The supernatant liquid, after removal of plasma euglobulin by dialysis, is not a potent source of enzyme.

THE EFFECT OF CALCIUM ION AND PLATELETS

In Tagnon's original preparation, the dog's plasma was recalcified before chloroform was added. It could be, therefore, contended that calcium was a possible reactant in the reaction he described. Similarly, the possibility of the presence of platelets in the plasma was not ruled out, and it might be argued by those adhering to the generally accepted theory of blood coagulation that thromboplastin of platelet origin was also involved. In the present studies, calcium ion was either removed by oxalate ion or rendered inactive by the presence of citrate ion; yet the reaction proceeded in the absence of that ion. To explore these facts further, many comparisons of fibrinolytic activity were made between recalcified and calcium ion free plasma, and platelet free and platelet rich plasma.⁵ No essential differences could be detected. It was, therefore, considered that this new enzyme system could be prepared from substances present in cell free plasma without the intervention of calcium ion. It was also considered that the proteolytic enzyme could be elaborated from some non-cellular plasma derivatives, such as either globulin substance or some substance associated with the plasma euglobulin.

The data of Table I also show a thrombic activity to be present in the preparation although platelets were removed and calcium ion absent. Further evidence was obtained by a study of the blood plasma enzyme obtained from a patient with thrombocytopenic purpura. Although the blood platelet count was only 2,000 per c.mm., the plasma enzyme was found in normal amounts.

Active enzyme preparations were also made by treating the portion of the plasma proteins precipitated between 20 per cent and 40 per cent saturation with ammonium sulphate, with chloroform and subsequently dialyzing in cellophane sacks.

DISCUSSION

The data presented indicate that when platelet free normal human plasma, free from active calcium ion, is treated with chloroform, a fraction of the plasma globulins appears to be changed or modified so that it exhibits the property of an

enzyme. This enzyme appears to have the property of lysing both fibrin and fibrinogen. The enzyme preparations contain neither prothrombin nor fibrinogen.

Tagnon (3) has shown that similar preparations made from recalcified dog's plasma had similar properties. He also showed that these preparations were able to transform prothrombin into thrombin without the presence of calcium. The presence of thrombic activity in most of our preparations from normal human plasma indicates that a similar transformation of prothrombin occurs. This concept is supported by the fact that chloroform plasma globulin preparations can coagulate solutions of fibrinogen. The presence of thrombic activity in a preparation from plasma containing no active calcium ion is interesting. This represents the second type of preparation from plasma in which such a situation has arisen. Parfentjev (17) described a pseudoglobulin fraction prepared from citrated or oxalated rabbit plasma, having a clot promoting activity. We were able to repeat these observations and also were able to prove the activity thrombic in nature (19). In these preparations, calcium ion was removed by addition of oxalate or citrate.

There is a marked difference, however, between the chloroform plasma globulin preparation and thrombin prepared by Parfentjev's procedure. The first has the power to clot oxalated plasma and fibrinogen solution and then rapidly to lyse the precipitated fibrin. Parfentjev's thrombin will coagulate fibrinogen instantly, but at no concentration will fibrinolysis occur, within the observation period of 24 hours.

Tagnon, using dog blood as a source of enzyme, stated that his preparation coagulated plasma, but not fibrinogen solution unless prothrombin were added. Using human blood, the chloroform preparation nearly always exhibited a slight thrombic activity. We have, however, by careful quantitation been able to find for all our preparations some concentration at which fibrinogenolysis occurs before coagulation by the thrombic activity can intervene. In both Tagnon's preparations and the present ones, thrombin always was produced as a result of chloroform action, but Tagnon was able to destroy the thrombic activity of his preparation by allowing the preparation to

⁵ Platelet rich plasma was obtained by adding to normal plasma the platelet suspension obtained when platelet free plasma was prepared.

stand, while we were unable to remove the last traces of it in our preparation by this method.

So far as the experiments on fibrinogen are concerned, we must for the present report that, dependent upon the concentration used, the following events occur. At low concentration of the chloroform plasma globulin, coagulation of fibrinogen occurs, while at higher concentration, the coagulation is followed by fibrinolysis, and at still higher concentrations, fibrinogenolysis occurs with no coagulation. The absence of fibrinogen can be demonstrated either by the addition of thrombin or by failure to obtain fibrinogen by heating or salting out methods.

Plasma and derivatives of plasma containing a high concentration of prothrombin definitely inhibit the proteolytic activity of the chloroform plasma preparation. This is indicated by the much higher concentration of the preparation required to effect lysis of the clot, and the still higher concentration required to remove fibrinogen by lysis prior to coagulation. The nature of this inhibition is under investigation at present. Jobling's experiments (5) on trypsin are of interest in this regard. This author concluded that an antitryptic material was removed from plasma by the action of chloroform. Unfortunately, his experiments do not preclude the possibility of augmentation of tryptic effect due to the elaboration of another but similar proteolytic enzyme by the action of chloroform on the plasma.

Fibrinolysis does occur in certain disease states subsequent to the coagulation of shed blood. Such instances, however, are rare except when bacterial contamination of the blood is present. Furthermore, the time required for such fibrinolysis is very long. In the present investigation, all preparations were made under sterile conditions and no bacterial contamination was found at the end of the incubation period. It is concluded, therefore, that the proteolytic activity was elaborated as a result of the chloroform treatment.

The inactive progenitor of the fibrinolytic factor present in plasma is uncertain. It can be stated, however, that the impure protein fraction, contained in globulin substance which has no proteolytic activity, can be activated by chloroform. The proteolytic activity of such a preparation is in no wise different from that obtained from whole plasma. Similarly, plasma euglobulin, equally in-

active as a proteolytic enzyme, can be activated by chloroform to develop marked fibrinolytic action. The supernatant liquid from the euglobulin preparation is not a source of significant amounts of the enzyme.

There has been, up to the present time, no indication of general species specificity. The proteolytic activity is present in chloroform plasma globulin preparations of human, dog, beef, rabbit, and swine blood. Horse blood is an exception insofar as preparations from such blood are only minimally potent.

In many respects, the chloroform plasma preparation, as reported here and earlier by Tagnon, resembles trypsin in its action on plasma, fibrinogen, and prothrombin. The rôle which chloroform plasma globulin plays in blood coagulation is not as yet clear. However, there is no doubt that the preparation can cause coagulation of oxalated blood and fibrinogen solution, with subsequent lysis.

SUMMARY

1. A method for preparing an active enzyme from blood plasma is given.
2. In higher concentrations, the enzyme can cause both fibrinogenolysis and fibrinolysis, in both plasma and pure fibrinogen solution.
3. Plasma preparations containing high concentrations of prothrombin inhibit the enzymatic activity.
4. Globulin substance and plasma euglobulin, which possess no proteolytic activity, can be converted into globulin fractions, possessing marked proteolytic activity, by the action of chloroform.
5. Removal of calcium and platelets has no demonstrable effect on the activity of the plasma enzyme.

BIBLIOGRAPHY

1. Nolf, P., Contribution à l'étude de la coagulation du sang. 5^e mémoire. La fibrinolyse. Arch. Internat. de Physiol., 1908, 6, 306.
2. Hougardy, A., La fibrinolyse. Arch. Internat. de Physiol., 1933, 36, 92.
3. Tagnon, H. J., The significance of fibrinolysis in the phenomenon of coagulation of blood. J. Lab. and Clin. Med., 1942, 27, 1119.
4. Barker, B., The enzymes of fibrin. J. Exper. Med., 1908, 10, 343.
5. Jobling, J. W., and Petersen, W., Nature of serum antitrypsin. J. Exper. Med., 1914, 19, 459.

6. Minot, G. R., The effect of chloroform on the factors of coagulation. *Am. J. Physiol.*, 1915, 39, 131.
7. Yamakawa, S., The autodigestion of normal serum through the action of certain chemical agents. *J. Exper. Med.*, 1918, 27, 689.
8. Eagle, H., and Harris, T. N., Studies in blood coagulation. V. The coagulation of blood by proteolytic enzymes (trypsin, papain). *J. Gen. Physiol.*, 1937, 20, 543.
9. Ferguson, J. H., and Erickson, B. N., Calcium and cephalin in relation to the clotting power of crystallin trypsin. *Proc. Soc. Exper. Biol. and Med.*, 1939, 40, 625.
10. Ferguson, J. H., The clotting of hemophilic plasma by thromboplastic enzyme. *Am. J. Physiol.*, 1939, 126, 669.
11. Tagnon, H. J., The significance of fibrinolysis in the mechanism of coagulation of blood. *Science*, 1942, 95, 334.
12. Lozner, E. L., and Taylor, F. H. L., The coagulation defect in hemophilia: Studies of the clot promoting activity associated with plasma euglobulin in hemophilia. *J. Clin. Invest.*, 1939, 18, 821.
13. Lozner, E. L., Kark, R., and Taylor, F. H. L., The coagulation defect in hemophilia: The clot promoting activity in hemophilia of Berkefelded normal human plasma free from fibrinogen and prothrombin. *J. Clin. Invest.*, 1939, 18, 603.
14. Kaplan, M. H., Tagnon, H. J., Davidson, C. S., and Taylor, F. H. L., The nature and properties of a proteolytic enzyme derived from plasma. *J. Clin. Invest.*, 1942, 21, 533.
15. Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., Study of coagulation defect in hemophilia and in jaundice. *Am. J. M. Sc.*, 1935, 190, 501.
16. Mellanby, J., Prothrombase, its preparation and properties. *Proc. Roy. Soc., s. B., London*, 1930, 107, 271.
17. Parfentjev, I. A., A globulin fraction in rabbit's plasma possessing a strong clotting property. *Am. J. M. Sc.*, 1941, 202, 578.
18. Taylor, F. H. L., Lozner, E. L., and Adams, M. A., The thrombic activity of a globulin fraction of rabbit plasma. *Am. J. M. Sc.*, 1941, 202, 585.
19. Patek, A. J., Jr., and Taylor, F. H. L., Hemophilia. II. Some properties of a substance obtained from normal human plasma effective in accelerating the coagulation of hemophilic blood. *J. Clin. Invest.*, 1937, 16, 113.
20. Mertz, E. T., and Owen, C. A., Imidazole buffer: Use in blood clotting studies. *Proc. Soc. Exper. Biol. and Med.*, 1940, 43, 204.
21. Seegers, W. H., Purification of prothrombin and thrombin. *J. Biol. Chem.*, 1940, 136, 103.

STUDIES ON BLOOD COAGULATION: THE NATURE AND PROPERTIES OF A PROTEOLYTIC ENZYME DERIVED FROM PLASMA^{1, 2, 3}

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Boston City Hospital, and the Department of Medicine,
Harvard Medical School, Boston)

(Received for publication May 28, 1942)

In the preceding paper (1) the preparation, by the action of chloroform on calcium and cell free plasma and subsequent precipitation of the globulins, of a globulin fraction having marked fibrinogenolytic and fibrinolytic properties was described. The present communication describes the chemical nature of these preparations. The preceding paper explains the activity of the chloroform plasma preparations on the basis of the presence, in them, of a proteolytic enzyme. The present communication offers evidence substantiating this assumption.

METHODS

Blood from the aorta of freshly stunned steers was collected in 20 per cent potassium oxalate solution. The final concentration of oxalate was 0.2 per cent. The plasma was removed by centrifuging. The plasma was then treated with chloroform. Chloroform plasma and its globulin derivatives were then prepared, as described in the preceding paper (1).

Determinations of pH were made by a glass electrode, non-protein nitrogen estimations by micro Kjeldahl methods, and viscosity determinations by the Ostwald viscosimeter at 37.5° C.

As substrates, fibrinogen, gelatin, and casein were used. Plasma was, in general, used as a source of fibrinogen and the lysis of precipitated fibrin followed by determinations of non-protein nitrogen at daily intervals. When

¹ Presented, in part, by M. H. Kaplan, as a thesis, to the Faculty of Arts and Sciences of Harvard College, in partial fulfillment of the requirements of the Degree of Bachelor of Arts in Honors.

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³ This study was aided, in part, by a grant "In recognition of Doctor Francis W. Peabody's services to the Foundation" by the Ella Sachs Plotz Foundation; and, in part, by a gift to Harvard University from the Smith, Kline and French Laboratories, Philadelphia, Pennsylvania.

⁴ Graduate Fellow of the Belgian American Educational Foundation, 1941-1942.

casein and gelatin were used as substrates, 2 per cent solutions of these proteins were employed.

In the non-protein nitrogen studies, an equal volume of the standard enzyme preparations was added to the substrate and the mixture adjusted to pH 7. Samples were removed at suitable intervals of time, and the progress of hydrolysis followed by the non-protein nitrogen produced.

For viscosimetric studies a 4 per cent gelatin solution was employed and the amounts of enzyme noted in the text were added. Both the gelatin and enzyme solutions were adjusted to the required pH, all particulate matter removed by centrifuging, and the solution brought to a temperature of 37.5° C. before mixing. The mixtures were transferred to the viscosimeters and the initial viscosity times recorded. No attempt was made to calculate absolute or relative viscosity, only the percentage change in viscosity time being of immediate interest. The viscosity was determined at 15 minute intervals.

Bacteriological cultures on all preparations showed that contamination by bacteria was absent in both plate and broth culture. One per cent chloroform was used as a bacteriostatic agent in the non-protein nitrogen experiments. As buffers, either phosphate or imidazole buffer (2) was used.

EXPERIMENTAL

The first observations of true proteolysis were made on plasma after the addition of chloroform. As has been stated before (1), the clot which initially forms undergoes lysis. The disappearance of this protein clot in 3 or 4 days was suggestive of proteolysis. When samples from the reaction vessels were removed and analyzed at daily intervals, an increase in non-protein nitrogen was found. No bacterial contamination was present. This, and many similar observations, indicate that during the preparation of the enzyme, hydrolysis of protein progressed. Similar proteolysis was observed when saline solutions of globulin substances were treated with chloroform (Figure 1).

Proteolysis of casein and gelatin. When the saline solution of the globulin fraction of steer plasma, obtained after the action of chloroform, was added to an equal volume of gelatin at pH 7, a marked increase in non-protein nitrogen occurred, indicating that digestion by

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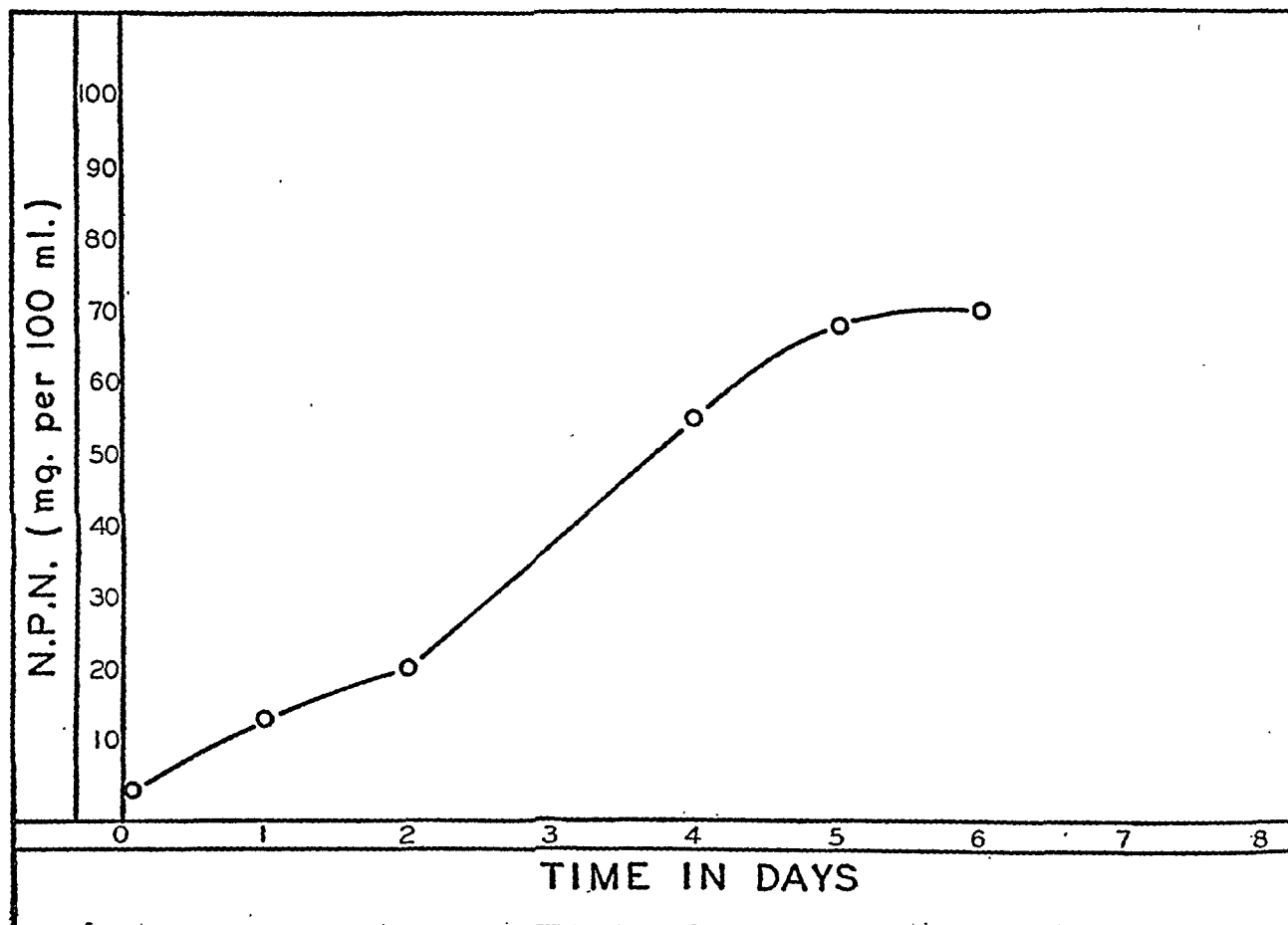


FIG. 1. INCREASE IN NON-PROTEIN NITROGEN FOLLOWING THE EXPOSURE OF PLASMA EUGLOBULIN TO THE ACTION OF CHLOROFORM

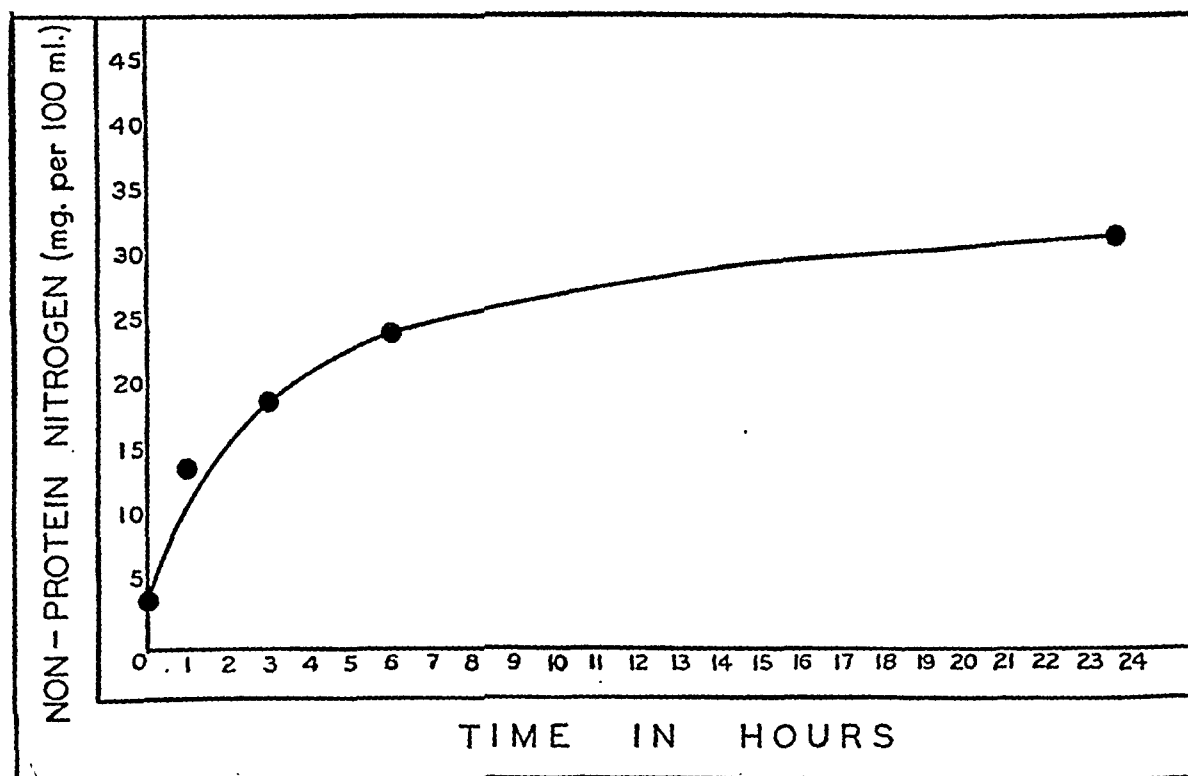


FIG. 2. HYDROLYSIS OF CASEIN BY THE PLASMA PROTEASE

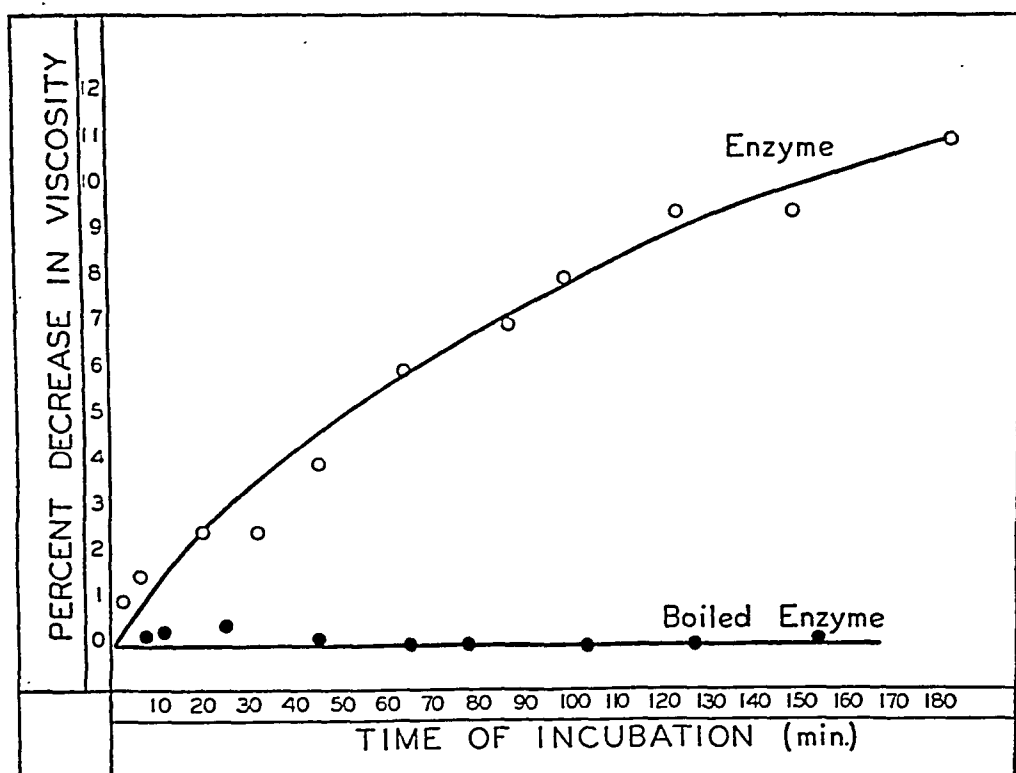


FIG. 3. THE EFFECT OF THE ACTION OF THE PLASMA ENZYME ON THE VISCOSITY OF A GELATIN SOLUTION

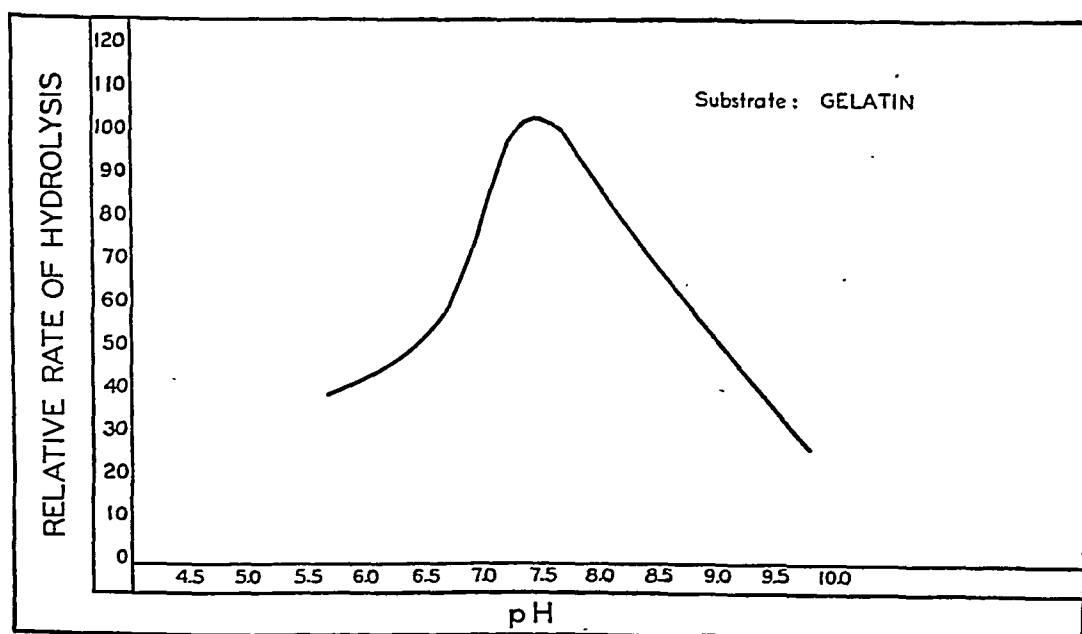


FIG. 4. THE EFFECT OF pH ON THE ACTIVITY OF THE PLASMA ENZYME

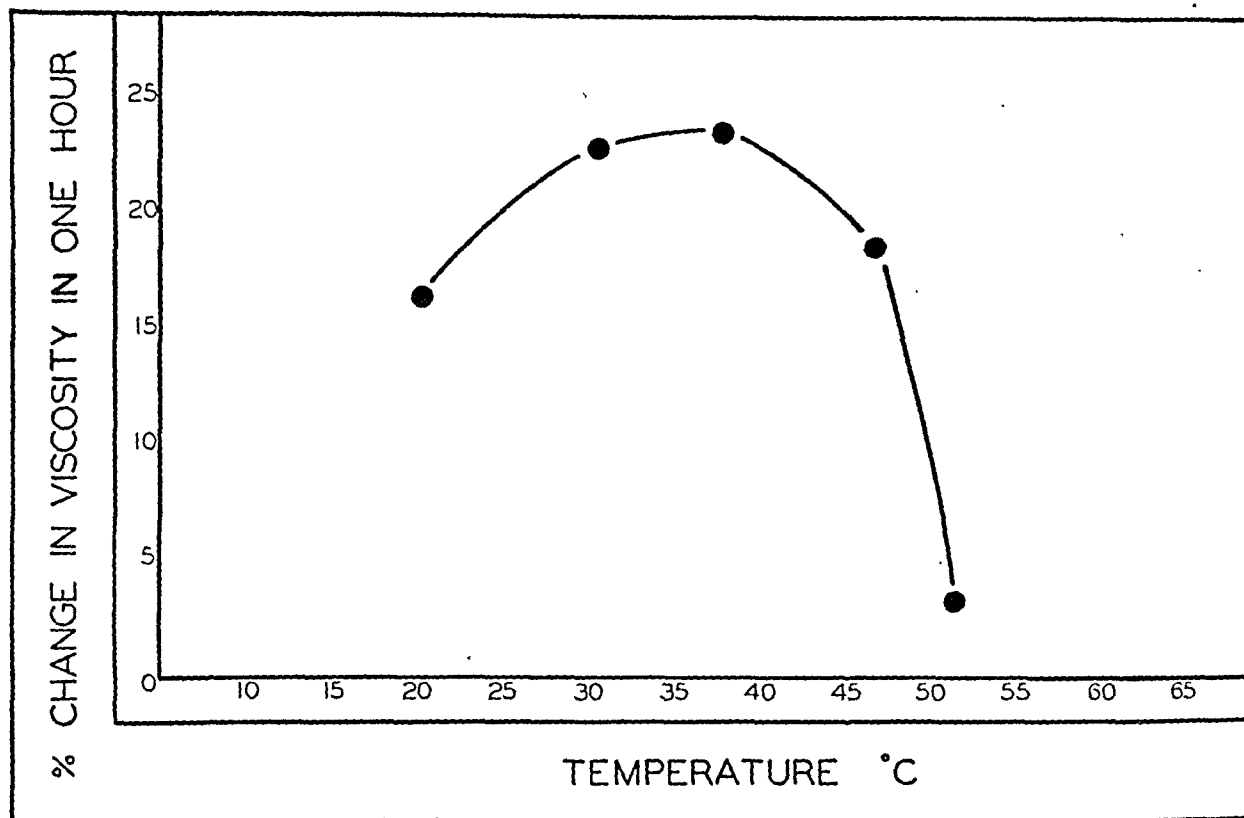


FIG. 5. THE EFFECT OF TEMPERATURE ON THE RATE OF HYDROLYSIS BY THE PLASMA PROTEASE

hydrolysis takes place (Figure 2). Entirely similar results were obtained when casein was used as a substrate.

Viscosimetric studies. To study the effect of pH and temperature on this enzyme, viscosimetric studies were employed. Figure 3 shows the effect of the active enzyme preparation on the rate of flow of a gelatin solution in a typical experiment. The lower curve is a control in which the enzyme solution was inactivated by boiling.

Since the enzyme preparations were obviously impure, there seemed no point in developing the reaction kinetics too far. The empirical equation of Schutz was applied to the reaction. Table I shows that the Schutz law holds remarkably well for the plasma enzyme.

The optimum pH of proteolysis by the plasma enzyme. Gelatin solutions, having an initial pH between 5.5 and 10, were made. The enzyme samples were adjusted to similar pHs and mixed. After each run, the pH was checked and the curve for change in viscosity time was drawn. The curve shown in Figure 4 was obtained by plotting the change in viscosity, 1 hour after the run was commenced, against the pH. It will be observed that the enzyme has an optimal pH at 7.4.

The effect of temperature on the plasma enzyme. As was shown in Figure 3, when a solution of the enzyme was boiled, its activity was destroyed. Figure 5 shows a composite of several enzyme curves giving the rates of viscosity change 1 hour after mixing at different temperatures from 2° to 50° C. The optimum temperature of the enzyme reaction was found to be approximately 36° C. There was no activity at temperatures above 50° C. The temperature coefficient (Q_{10}) for the 10 degree interval between 20° and 30° C. was 1.5. The tempera-

ture coefficient was obtained by determining the ratio of the reaction velocities at 20° and 30° C. This coefficient was within the range found for most proteolytic enzymes. Trypsin alone has a higher temperature coefficient.

DISCUSSION

The foregoing evidence indicates that a true proteolytic enzyme can be prepared from oxalated or citrated steer plasma by the addition of chloroform. This proteolytic activity is associated with the globulin fraction of the plasma proteins; after treatment with chloroform.

As early as 1903, Delezenne and Pozerski (3) reported that normal serum treated with chloroform had a lytic effect on gelatin. Abderhalden (4), knowing that normal serum had no such proteolytic effect, dismissed this finding as being due to the action of chloroform on the leukocytes. Barker (5) believed that the fibrinolysis of clots, which he also observed, was due to entrapped mononuclear cells. In all this work, however, the manipulations required might well justify Abderhalden's criticism of an intracellular source of the enzyme. The first investigator to work with cell free plasma was Hedin (6) who in 1904, showed that it could digest both casein and gelatin but not coagulated egg albumin. Unfortunately,

Hedin's preparations were very weakly active, and precise data on the properties of the enzyme were not obtainable.

TABLE I

Application of the Schulz rule to the plasma protease

Time of incubation in hours	η change in viscosity	$K = \frac{\eta}{\sqrt{t}}$
2	9.0	6.4
4	15.3	7.7
6	20.1	8.3
8	24.3	8.6
10	28.0	8.8
12	30.9	8.9
14	33.0	8.8
16	34.5	8.6
18	35.4	8.3
20	36.0	8.0

The present data indicate that the active enzyme is a protein capable of lysing fibrin, fibrinogen, casein, and gelatin. Lysis is accompanied by an increase in split protein products and also by a diminution of the viscosity of gelatin. The enzyme is thermolabile, being destroyed by boiling, and having an optimal temperature *in vitro* of 36° C. Its optimal pH is between 7.4 and 7.9.

Schmitz (7), in 1936, was able to isolate from plasma an enzyme capable of producing slight increases in non-protein nitrogen when added to substrates of casein and gelatin. It is interesting that his data for optimum pH agree fairly well with our own, although the activity of his preparations was much less.

The plasma enzyme resembles trypsin although identification of the new enzyme with trypsin is not at present possible. It is probably not of pancreatic origin since derivatives of depancreatized dog plasma have been shown to be quite satisfactory for the formation of the enzyme (8).

The data of this communication deal with preparations of steer blood. Similar preparations from human and swine plasma were, by the methods described herein, equally active as proteolytic enzymes. However, horse plasma has been found deficient as a source of the enzyme.

CONCLUSIONS

(1) When chloroform acts on oxalated or citrated steer plasma and plasma globulins subsequently precipitated, these globulin fractions have the properties of an enzyme.

(2) This enzyme is capable of digesting fibrinogen, fibrin, gelatin, and casein as indicated by the progressive formation of non-protein nitrogen from the substrates. The non-protein nitrogen studies were confirmed in the case of gelatin by viscosimetric studies.

(3) The enzyme has an optimal pH of 7.4 at 37.5° C.

(4) The enzyme is destroyed by boiling and exhibits its optimal activity at a temperature of 36° C. at pH 7.

BIBLIOGRAPHY

1. Tagnon, H. J., Davidson, C. S., and Taylor, F. H. L., Studies in blood coagulation: A proteolytic enzyme prepared from calcium and platelet free normal human blood plasma. *J. Clin. Invest.*, 1942, 21, 525.
2. Mertz, E. T., and Owen, C. A., Imidazole buffer: Use in blood clotting studies. *Proc. Soc. Exper. Biol. and Med.*, 1940, 43, 204.
3. Delezenne, C., et Pozerski, E., Action du sérum sanguin sur la gélatine en présence de chloroforme. *Compt. rend. Soc. de biol.*, 1903, 4, 327.
4. Abderhalden, E., *Abwehrfermente, das Auftreten blutfremder Substrate und Fermente im tierischen Organismus unter experimentellen, physiologischen, und pathologischen Bedingungen.* Springer, Berlin, 1914, Fourth Edition.
5. Barker, B. I., The enzymes of fibrin. *J. Exper. Med.*, 1908, 10, 343.
6. Hedin, S. G., On the presence of a proteolytic enzyme in the normal serum of the ox. *J. Physiol.*, 1904, 30, 195.
7. Schmitz, A., Über die Proteinase des Fibrins (1). *Ztschr. f. physiol. Chem.*, 1936, 244, 89.
8. Tagnon, H. J. (Unpublished observations.)

THE DIGITAL BLOOD FLOW, ARTERIAL PRESSURE, AND VASCULAR RESISTANCE IN ARTERIAL HYPERTENSION AND IN CORONARY THROMBOSIS

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Methods for the measurement of the digital blood pressure and flow have been described in a previous report (1). The calculation of the blood flow from calorimetric observations has since been modified, and a method developed for calculating the digital vascular resistance. With these methods, studies have been made on normal subjects and on patients with various types of hypertension. Since a decrease in cardiac output had been found after coronary artery ligation in dogs (2), investigations were also made on patients with coronary occlusion in order to determine whether a consistent change in the digital circulation could be demonstrated.

METHOD

All measurements were made under standardized conditions. The patient was resting in bed and the measurements were made after warming the body, by placing one arm in water at a temperature of from 43° to 45° C. until sweating became generalized. After this procedure, reproducible digital blood flow measurements are obtainable in the same individual, whether normal or abnormal. Whether the degree of vasodilatation thus obtained represents a complete release of sympathetic tone, however, is still controversial.

The digital blood flow was measured by the calorimetric method (1). According to Stewart (3), the specific heat of normal blood is nearer 0.9 than 1.0. The specific heat of tissue, on the other hand, is approximately 0.8. The accuracy of the method was therefore slightly increased by substituting 0.9 for 1.0 as the specific heat of the blood, and by adding the hydrothermic equivalent of the immersed finger tip (volume multiplied by 0.8) to the other hydrothermic equivalents.

The exact relationships between blood flow, blood pressure, blood viscosity, and frictional resistance of the vascular bed have not yet been clearly delineated. For practical purposes, however, the relationships proposed by Böger and Wezler (4) and by Bazett, Cotton, Laplace and Scott (5) may be applied to the digital circulation. According to these workers, if F is the blood flow, P , the effective mean arterial pressure, and R , the so-called

peripheral vascular resistance, then,

$$F \propto P;$$

and

$$F \propto \frac{1}{R};$$

hence,

$$F = \frac{P}{R} K;$$

and

$$R = \frac{P}{F} K.$$

On close analysis, R depends chiefly on the size of the vascular bed and on the viscosity of the blood. It has been shown that the effective viscosity varies very little with erythrocyte concentrations in the normal range of variation (6). Since our measurements were made upon patients whose erythrocyte counts varied from four to five million per c. mm., the viscosity factor may be assumed to be relatively fixed and variations in R to be caused chiefly by variations in the size of the vascular bed, especially the cross-sectional area.

In determining the peripheral vascular resistance, R , of the entire systemic circulation, Bazett, Cotton, Laplace and Scott (5) assigned an arbitrary value of 3.0 to K in order to bring the normal units of resistance approximately to 100. The corresponding value for K in the digital circulation is 0.3, since F is expressed in cc. per sq. cm. per minute, instead of in liters per sq. meter per minute. Until the exact physical relationships governing the peripheral circulation are worked out, such arbitrary units are preferable, for comparative measurements, to the physical units proposed by Böger and Wezler (4).

The digital vascular resistance, R , therefore, is calculated from the equation,

$$R = \frac{P}{F} K.$$

The value for K is 0.3. F is the digital blood flow in cc. per sq. cm. per minute as measured by the calorimetric method. P is the effective mean digital arterial pressure which is obtained by subtracting the venous pressure from the arithmetic mean of digital systolic and diastolic pressures, as measured with the Gaertner capsule (7, 8). For practical purposes the arithmetic mean is an adequate approximation of the true mean arterial pressure. The venous pressure is arbitrarily assumed to be 6 mm. of mercury in the absence of congestive heart failure. In many of the cases of coronary thrombosis, the actual ante-

cubital venous pressure was determined by the direct method and in most, found to be normal. In the occasional case in which it was found elevated, the actual value was substituted for 6 mm. Since measurements of digital arterial pressure were made approximately at heart level, the hydrostatic factor could be disregarded.

Twelve normal subjects, thirty patients with various types of hypertension, and twenty-six cases of coronary occlusion, were studied with these methods. Many of the patients with coronary occlusion had preëxisting hypertension. In some, the antecedent hypertension was verifiable from previous records or from the history, whereas in the remainder, the previous blood pressures were either normal or unknown.

RESULTS

Normal subjects

The results in twelve normal subjects are presented in Table I. With the new modifications in method, the normal range of variation in digital blood flow was from 0.23 to 0.34 cc. per sq. cm.

TABLE I
Normal

Patient	Age and sex	Preliminary brachial blood pressure	After body warming			
			Brachial blood pressure	Digital blood pressure	Digital blood flow	Digital vascular resistance
		mm. Hg	mm. Hg	mm. Hg	cc. per sq. cm. per minute	units
P. W.	22 M	110/75	102/74	88/40	0.27	63
A. S. H.	31 M	125/86	130/85	106/50	0.23	93
A. R.	25 M	118/88	112/82	84/70	0.31	69
W. P.	23 M	120/85	118/85	102/64	0.24	96
A. F. M.	30 M	122/84	118/90	96/60	0.34	63
A. L.	24 M	118/75	108/74	104/64	0.31	75
A. M.	45 M	112/75	114/72	108/68	0.32	75
M. M.	31 M	125/84	115/86	98/55	0.31	69
A. K.	50 M	122/86	105/72	86/60	0.33	61
P. S. C.	45 F	102/72	104/68	96/64	0.30	75
G. J. C.	51 M	130/85	125/78	108/70	0.31	81
M. C. M.	25 F	110/72	104/74	88/50	0.26	72

per minute. The average was 0.29 cc. \pm a standard deviation of 0.03 cc. The average arithmetic mean digital arterial pressure was 78 mm. of mercury \pm a standard deviation of 7 mm. The average arithmetic mean brachial arterial pressure was 96 mm. of mercury \pm a standard deviation of 7 mm. The average digital vascular resistance was

74 units \pm a standard deviation of 11 units. The normal range of variation was from 63 to 96 units.

Arterial hypertension

The results in thirty cases of hypertension are presented in Table II. Eight apparently normal subjects had elevated blood pressure before body warming. In these cases, the hypertension may be called neurogenic since it subsided after body warming, no increase in fixed resistance being demonstrable. In two of these cases (J. J. and L. L.), the vascular resistance was on the borderline, and it was impossible to be sure that these patients did not have early fixed hypertension. In one case of pheochromocytoma, the elevated preliminary blood pressure subsided promptly after warming the body and no increase in fixed resistance could be demonstrated.

In three cases of early essential hypertension in young people, the blood flow was definitely below the lower limit of normal and the resistance elevated out of proportion to the rise in blood pressure. Similar observations were made in three cases of acute glomerulonephritis. In two of these cases the increase in resistance was reversible, since both the blood flows and blood pressures became normal after the nephritis subsided.

In nine cases of essential hypertension of several years duration, in one case of chronic glomerulonephritis, and in one case of toxemia of pregnancy, the digital blood flows were within normal limits, and the resistances elevated in proportion to the blood pressure levels. In several of these cases, there was a superimposed complement of neurogenic hypertension, since the preliminary blood pressures dropped to less elevated levels after warming the body.

In three of the four cases of malignant hypertension, the digital blood flow was decreased and in the fourth, it was at the lower limit of normal. In these cases, the digital vascular resistance was elevated out of proportion to the increase in blood pressure.

Coronary occlusion

The results in twenty-six cases of coronary occlusion are presented in Table III. In ten of the cases of acute myocardial infarction, both the clin-

TABLE II
Hypertension

Patient	Age and sex	Diagnosis	Preliminary brachial blood pressure	After body warming			
				Brachial blood pressure	Digital blood pressure	Digital blood flow	Digital vascular resistance
			mm. Hg	mm. Hg	mm. Hg	cc. per sq. cm. per minute	units
B. B.	20 F	Neurogenic hypertension	145/100	135/94	110/76	0.32	81
B. R.	27 M	Neurogenic hypertension	148/95	132/86	116/60	0.34	72
P. W.	24 M	Neurogenic hypertension	138/88	128/80	102/60	0.32	72
D. S.	18 M	Neurogenic hypertension	140/94	125/84	96/52	0.26	78
A. D.	23 M	Neurogenic hypertension	148/88	152/72	112/75	0.31	84
F. C.	40 F	Neurogenic hypertension	140/86	142/76	102/52	0.29	75
J. J.	27 M	Neurogenic hypertension	145/88	125/75	88/70	0.25	96
L. L.	24 M	Neurogenic hypertension	160/95	135/88	125/75	0.28	100
M. M.	17 F	Pheochromocytoma	157/120	145/70	120/80	0.32	90
I. B.	21 F	Acute glomerulonephritis	140/88	130/85	116/82	0.21	132
J. N.	38 M	Acute glomerulonephritis	180/118	158/102	155/84	0.19	180
		Recovered	134/80	134/84	110/82	0.27	99
E. H.	32 F	Acute glomerulonephritis	185/120	190/115	160/115	0.17	231
		Recovered	130/75	128/74	106/78	0.31	83
R. M.	21 M	Essential hypertension	155/80	155/70	105/75	0.21	120
H. B.	22 M	Essential hypertension	165/80	135/68	120/75	0.20	122
E. K.	34 M	Essential hypertension	175/120	170/115	140/112	0.19	189
P. L.	20 M	Essential hypertension	180/105	155/95	120/92	0.26	115
G. B.	28 F	Essential hypertension	182/130	182/130	155/122	0.25	156
A. C.	35 M	Essential hypertension	160/104	162/102	128/100	0.25	137
L. T.	57 F	Essential hypertension	210/120	212/118	175/68	0.26	141
A. F.	58 F	Essential hypertension	175/96	178/90	110/94	0.23	126
A. S.	41 F	Essential hypertension	174/96	160/95	136/96	0.26	126
P. S.	47 F	Essential hypertension	235/125	212/134	160/115	0.25	156
E. S.	38 F	Essential hypertension	230/155	226/142	190/140	0.30	159
L. L.	35 M	Essential hypertension	165/108	160/115	118/100	0.25	129
J. S.	24 M	Chronic glomerulonephritis	165/115	158/95	148/108	0.26	141
L. N.	35 F	Toxemia of pregnancy	185/108	195/102	182/94	0.32	123
I. G.	56 F	Malignant hypertension	250/130	238/125	216/130	0.21	240
J. P.	48 F	Malignant hypertension	255/135	240/134	212/112	0.23	204
D. B.	42 M	Malignant hypertension	220/150	215/155	198/154	0.14	363
I. R.	48 M	Malignant hypertension	238/150	245/135	205/112	0.15	306

ical course and the electrocardiographic changes were typical. In nine of these cases the digital blood flow was below the lower limit of normal and in the remaining case, within the normal range.

In five cases of acute myocardial infarction, the clinical course of the disease was characteristic but the electrocardiographic findings were equivocal, either because the patient was seen several weeks after the onset of symptoms, or because of associated electrocardiographic changes, such as intra-ventricular block. In each of these cases, the digital blood flow was below the lower limit of normal.

In five cases, the clinical course was atypical despite progressive changes in the electrocardio-

gram. No definite diagnosis could be made in these cases although they were suggestive of either coronary occlusion or coronary insufficiency. The digital blood flow was found to be decreased in three of these cases and normal in two.

In three cases of coronary occlusion, studied several months or more after the acute attack, the digital blood flow was normal in all three.

No cases of acute coronary occlusion were studied in the first few days after the onset of symptoms, when the blood pressure is said to rise in some cases because of compensatory vasoconstriction (9). In the more stabilized phase in which these cases were studied, the digital vascular resistance was generally in accord with the level of the blood pressure prior to the acute at-

TABLE III
Coronary occlusion

Patient	Age and sex	Clinical course	Electro-cardiogram	Date of onset of symptoms	Date of observations	Previous brachial blood pressure	Pro-liminary brachial blood pressure	After body warming			
								Brachial blood pressure	Digital blood pressure	Digital blood flow	Digital vascular resistance
B.S.	55 M	Typical; second attack	Progressive change	May 10, 1941	June 6, 1941	230/?	142/90	130/86	130/85	cc. per sq. cm. per min.	units
B.M.	63 M	Typical	Progressive change	May 13, 1941	June 20, 1941	Unknown	120/88	112/85	88/72	0.22	102
S.P.	52 F	Typical	Progressive change	July 2, 1941	August 4, 1941	High	126/80	126/90	118/102	0.27	117
E.S.	48 F	Typical	Progressive change	July 25, 1941	August 23, 1941	200/?	160/102	164/102	140/78	0.18	180
A.O.	52 M	Typical	Progressive change	August 10, 1941	September 19, 1941	140/86	118/84	116/76	110/76	0.14	186
S.W.	59 M	Typical	Progressive change	September 2, 1941	September 30, 1941	Unknown	135/95	108/82	98/68	0.12	192
J.K.	47 M	Typical	Progressive change	September 8, 1941	October 8, 1941	132/92	116/72	110/74	102/64	0.16	144
A.B.	60 F	Typical	Progressive change	September 30, 1941	October 25, 1941	Unknown	105/85	88/68	72/60	0.20	90
H.M.	63 M	Typical	Progressive change	November 22, 1941	December 6, 1941	Unknown	116/78	118/76	100/64	0.18	127
N.A.	62 M	Typical; second attack	Progressive change	October 12, 1941	November 16, 1941	Normal	100/72	98/74	70/48	0.16	89
B.L.	55 F	Typical; second attack	Equivocal	July 9, 1941	July 23, 1941	190/?	138/100	138/94	125/86	0.17	177
M.N.	50 F	Typical	Equivocal	August 9, 1941	September 18, 1941	160/110	125/88	128/88	104/90	0.18	153
A.G.	87 M	Typical	Equivocal	August 27, 1941	October 2, 1941	Unknown	120/90	98/76	96/60	0.22	99
S.D.	63 M	Typical	Equivocal	October 24, 1941	November 17, 1941	150/90	135/65	124/68	106/64	0.16	147
P.N.	43 M	Typical	Equivocal	November 8, 1941	November 17, 1941	Unknown	135/90	116/74	88/70	0.12	183
R.S.	58 F	Atypical	Progressive change	May 9, 1941	June 10, 1941	180/?	160/80	150/85	118/92	0.28	108
G.L.	58 F	Atypical	Progressive change	July 9, 1941	August 1, 1941	180/?	165/75	145/72	102/70	0.26	93
M.H.	40 F	Atypical	Progressive change	November 17, 1941	November 29, 1941	High	150/92	138/76	108/74	0.21	121
M.M.	65 F	Atypical	Progressive change	November 27, 1941	December 10, 1941	High	155/68	142/50	96/72	0.14	163
S.B.	52 F	Atypical	Progressive change	December 4, 1941	December 20, 1941	190/85	158/88	148/78	124/86	0.16	186
S.V.	63 M	Atypical; second attack	Equivocal	October 2, 1941	October 31, 1941	180/100	150/84	150/85	114/72	0.23	114
J.G.	50 M	Atypical; second attack	Equivocal	October 31, 1941?	November 11, 1941	Unknown	110/68	110/80	98/56	0.29	74
D.R.	39 M	Atypical	Equivocal	October 1, 1941?	November 3, 1941	150/?	130/88	142/94	118/84	0.18	159
S.K.	48 M	Typical	Progressive change	July 5, 1940	November 24, 1941	Normal	125/88	116/84	92/78	0.34	69
S.S.	64 M	Typical	Progressive change	May 6, 1936	November 14, 1941	140/90	155/95	138/90	115/80	0.24	114
B.M.	55 M	Atypical	Progressive change	February 19, 1941	November 16, 1941	150/110	142/98	138/84	120/82	0.26	111

tack, where that could be determined. The resistance was elevated in twenty cases and normal in six. In seventeen of the twenty cases in which it was elevated, the previous blood pressure was known to be high, either from the hospital records or from the patient's statement. The correlation between the level of the resistance and the level of the previous blood pressure was accurate in some cases and only fairly accurate in others, a discrepancy ascribable to errors in measuring blood pressure and to fluctuations in the neurogenic complement of the hypertension. Of the six cases with normal digital vascular resistance, the previous blood pressure was known to be normal in two and unknown in three. In the remaining case (G. L.), the antecedent blood pressure was known by the patient to be high. This patient, however, was psychoneurotic and her blood pressure fluctuated widely under observation. The basal level, therefore, may well have been normal in this case.

In all the cases of acute myocardial infarction in which the digital blood flow was decreased, the brachial and digital blood pressures were

below the level of the previous blood pressures and below the level to be expected from that of the vascular resistance. Many of these blood pressures, however, at face value, appeared normal. It is apparent, therefore, that the decrease in blood flow was proportional to the decrease in blood pressure, the vascular resistance remaining essentially unchanged. In those cases, on the other hand, in which the digital blood flow was normal (doubtful and old cases), the brachial and digital blood pressure levels were in accord with previous blood pressure levels and with the vascular resistances.

COMMENT

Discussion of methods

Many attempts have been made to measure the peripheral resistance in normal subjects and in patients with hypertension. These studies have been of two main varieties. In the first, the general systemic peripheral resistance is calculated from the left ventricular output and the brachial arterial blood pressure; whereas in the second,

the local vascular resistance in a part of the systemic circulation, such as the forearm or hand, is calculated from the blood pressure in the local artery and the blood flow per unit of tissue.

Calculation of the general systemic vascular resistance from the cardiac output and the aortic blood pressure depends upon accurate measurement. The relatively accurate Fick (10) and acetylene (11) methods are time-consuming and too disturbing for use in seriously ill patients. The more indirect methods, such as the ballistocardiographic technique (12) and the methods of Böger and Wezler (4) and of Bazett, Cotton, Laplace and Scott (5), are fairly accurate when applied to normal subjects but introduce considerable error, especially in the abnormal patient. The use of the brachial arterial blood pressure to indicate pressure in the ascending aorta may also introduce some error, especially in the presence of circulatory disturbances. In addition, as pointed out by Wiggers (13), the measurement of systemic vascular resistance by these techniques includes the variable of resistance in the large arteries as well as the more important variable of arteriolar resistance. Although it is true that it is the total peripheral resistance which determines the level of the blood pressure rather than the resistance in the terminal arterioles alone, changes in the latter are agreed to be of more fundamental importance in hypertension and might therefore be better measured separately.

The methods hitherto employed to measure local blood flow, blood pressure, and vascular resistance have also been unsatisfactory. One of the methods ordinarily used to measure blood flow quantitatively is the plethysmographic. The most important difficulty encountered with this method has been the enormously wide normal range of variation in blood flow per unit of tissue. In the plethysmographic studies of Prinzmetal and Wilson (14), this range was particularly wide since these authors did not take the precaution of shutting off the more labile circulation of the hand (15). The normal resting blood flow varied from 1.75 to 7.0 cc. per 100 cc. of tissue per minute; whereas, after release of sympathetic tone by body warming, it varied from 2.3 to 10.1 cc. per 100 cc. per minute. Abramson (16) took the precaution of excluding hand blood flow but measured only the resting blood flow, and con-

cluded that it was greater in patients with hypertension than in normal subjects. The normal range of variation was from 0.9 to 3.3 cc. per 100 cc. per minute, and in hypertension from 1.4 to 4.0 cc. per 100 cc. per minute. The wide normal range of variation in resting blood flow, due, at least in part, to variations in vasomotor nerve tone, makes it very difficult to evaluate deviations from the normal because of overlapping. Wilkins and Eichna (17) found a normal range of from 2.3 to 7.0 cc. per 100 cc. per minute, even after taking the precaution of warming the body and of measuring only forearm blood flow by shutting off the circulation to the hand. In the foot, Stead and Kunkel (18) found the normal range to be from 14.0 to 34.0 cc. per 100 cc. per minute after vasodilatation by body warming and local heat. These wide normal ranges of variation are probably attributable to the limitations of the Hewlett and van Zwaluwenburg technique (19) of measuring blood flow plethysmographically after venous occlusion. The possible sources of error are incomplete venous obstruction because of venous flow through the bones, and variable proportion of bone to muscle and skin mass. In addition, the rising venous pressure after venous obstruction produces a progressively smaller effective mean arterial pressure even in the presence of an unchanging brachial arterial blood pressure. Since it is extremely difficult technically to measure this effect, another variable error is introduced, especially if vascular resistance is to be measured. Similar objections can be made to the digital plethysmograph (20) except that the technical difficulties here are even more formidable.

Pickering (21) considered heat elimination in the hand calorimeter to be proportional to blood flow, but also found a wide normal range of variation even after body warming. The most important sources of error in the hand calorimeter are the discrepancy between venous blood temperature and calorimeter temperature (22), and the use of volume instead of surface for tissue units (23).

In the finger tip calorimeter the variation between actual venous blood temperature and calorimeter temperature is minimal (1), probably because almost the entire blood flow is through the surface vessels. The ease of measuring the surface of the finger tip and the expression of the

digital blood flow in terms of surface rather than volume also eliminate an important source of error. With this method, therefore, the normal range of variation in blood flow (0.23 to 0.34 cc. per sq. cm. per minute) is much narrower than with any of the other methods. The blood pressure to the terminal digit, moreover, can be measured directly and need not be inferred from the brachial blood pressure. In addition, there are no abnormal constrictions and the effective mean pressure can be calculated from the arterial pressure and the normal or abnormal venous pressure.

The digital calorimetric method for measuring blood flow, together with all local methods, has the disadvantage of measuring the status of a small portion of the systemic circulation rather than that of the circulation in general. The results are therefore difficult to interpret in the presence of conditions affecting the local circulation, such as scleroderma or clubbing. Another disadvantage is the measurement of the circulation under unusual conditions, namely, after vasodilatation by body warming. Normal digital blood flow, however, is defined by measurement under the same circumstances, and since the normal range of variation in blood flow is narrowed extremely by preliminary body warming, this procedure becomes indispensable for interpreting deviations from the normal. Without it, the overlapping of the normal range by the abnormal would make it impossible to distinguish one from the other. It is sufficient, therefore, for comparative purposes if the digital circulation studied in this way mirrors the systemic circulation in general, which, in the absence of local disease of the fingers, we have reason to believe it does.

Discussion of results

It can be seen from Table II that after vasodilatation by body warming, the digital circulation of patients with neurogenic elevations of blood pressure is indistinguishable from that of normal subjects. In fact, it is probable that these patients are entirely normal, except for a tendency to neurogenic vasoconstriction in response to certain stimuli. By measuring the digital circulation after body warming, it is possible to distinguish a neurogenic and probably transient elevation of blood pressure from one which is fixed. Since

neurogenic vasoconstriction may also be observed in cases of true essential or renal hypertension, study of the digital circulation in these cases makes it possible to determine how much of the elevation of blood pressure is neurogenic and how much is fixed.

It is difficult to draw definite conclusions from the small number of observations made in acute glomerulonephritis and in early essential hypertension. The decrease in blood flow found in these cases, however, is evidence in favor of the view that an increase in fixed resistance is the primary process in these diseases. Hypertrophy of the heart may lag behind and eventually cause a further rise in blood pressure and a restoration of the blood flow to within normal limits in the more advanced stages of essential or renal hypertension. In the malignant phase, the hypertrophied heart can probably no longer keep pace with the further increase in resistance by equivalent elevation of the blood pressure, and the blood flow again decreases. It is apparent, therefore, that only in established essential or renal hypertension is the blood pressure an adequate index of the peripheral resistance. In the acute or malignant phase, the actual resistance may be considerably greater than is indicated by the blood pressure level. It should also be noted that the increase in fixed resistance in acute glomerulonephritis is reversible.

From Table III, it is apparent that a decreased digital blood flow is a consistent finding in the first six weeks after a coronary occlusion. This phenomenon was observed in several cases in which the electrocardiograms were equivocal, although the clinical course was characteristic of coronary occlusion. In the doubtful cases, in which both the clinical course was atypical and the electrocardiograms inconclusive, it is probable that the demonstration of a decreased digital blood flow indicated the presence of an acute myocardial infarct.

Since it is possible, on the other hand, for the blood flow to be within normal limits in an occasional case of acute coronary occlusion, the demonstration of a normal digital blood flow cannot be considered absolute evidence of the absence of acute myocardial infarction. In addition, a decrease in digital blood flow is demonstrable only

the first six weeks, or perhaps in some cases, a few months after the onset of the acute occlusion; in cases studied several months or more after the acute coronary thrombosis, the digital blood flow was within normal limits. It should also be pointed out that a decrease in digital blood flow is not pathognomonic of coronary occlusion. Such decreases occur in acute and malignant hypertension, in various peripheral vascular diseases and also in systemic conditions associated with shock. Studies of the digital circulation alone in such cases would be difficult to interpret, although they might render valuable information, especially if concomitant measurements of blood volume and other aspects of the circulation were made. The demonstration of decreased digital blood flow can be helpful, however, in the differentiation of a genuine case of coronary thrombosis from angina pectoris.

The correlation between the digital vascular resistance as measured after acute coronary occlusion and the antecedent blood pressure level makes it possible to determine the presence or absence of preëxisting hypertension. The determination of the digital vascular resistance thus offers a method of measuring the extent of the fundamental vascular abnormality in hypertension, even when complicating factors have reduced the blood pressure.

From these studies, the probable mechanism of the circulatory changes produced by an acute coronary occlusion may be visualized as follows: A decrease in myocardial contractile force is produced by the infarction. This operates against an unchanged vascular resistance or a resistance increased by compensatory vasoconstriction to bring about a decrease in arterial blood pressure. The decrease in arterial blood pressure is not, in other words, caused by a decrease in resistance, but largely by a decrease in the cardiac driving force. The decrease in arterial blood pressure, together with such compensatory vasoconstriction as takes place, causes a decrease in systemic blood flow, venous return, and cardiac output. This produces a further decrease in blood pressure. The process, therefore, is probably one of progressive deceleration of the circulation, ultimately balanced at a lower equilibrium by those forces which tend to keep the blood flow normal.

SUMMARY AND CONCLUSIONS

1. Methods for calculating the digital blood flow from calorimetric observations have been modified and a method developed for calculating digital vascular resistance.

2. After vasodilatation produced by warming the body, normal digital blood pressure, blood flow, and vascular resistance were found in patients with neurogenic elevations of blood pressure. A consistent increase in digital vascular resistance and a normal digital blood flow were demonstrated in established essential or renal hypertension. In acute and in malignant hypertension, the digital blood flow may be decreased, and the vascular resistance increased out of proportion to the elevation in blood pressure.

3. Decrease in digital blood flow and blood pressure, and unchanged vascular resistance were demonstrated in cases of acute coronary occlusion, whether the antecedent blood pressure was normal or elevated.

This opportunity is taken of acknowledging the cooperation of Drs. B. S. Oppenheimer, G. Baehr, E. Moschowitz, and E. P. Boas in making these cases available for study, and of Dr. A. M. Master, in whose department some of these studies were carried out.

BIBLIOGRAPHY

1. Mendlowitz, M., Measurements of blood flow and blood pressure in clubbed fingers. *J. Clin. Invest.*, 1941, 20, 113.
2. Mendlowitz, M., Schauer, G., and Gross L., Hemodynamic studies in experimental coronary occlusion. III. Denervated heart experiments. *Am. Heart J.*, 1937, 14, 21.
3. Stewart, G. N., Blood flow in the hand, normal and pathological. *Harvey Lectures*, 1912, 8, 86.
4. Böger, A., and Wezler, K., Die Bestimmung des arteriellen Gesamtwiderstandes am Menschen. *Arch. f. exper. Path. u. Pharmacol.*, 1937, 186, 43.
5. Bazett, H. C., Cotton, F. S., Laplace, L. B., and Scott, J. C., The calculation of cardiac output and effective peripheral resistance from blood pressure measurements with an appendix on the size of the aorta in man. *Am. J. Physiol.*, 1935, 113, 312.
6. Whittaker, S. R. F., and Winton, F. R., The apparent viscosity of blood flowing in the isolated hind limb of the dog and its variation with corpuscular concentration. *J. Physiol.*, 1933, 78, 339.
7. Gaertner, G., Über einen neuen Blutdruckmesser (Tonometer). *Wien. klin. Wchnschr.*, 1899, 12, 696.

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neurogenic vasoconstriction may also be observed in cases of true essential or renal hypertension, study of the digital circulation in these cases makes it possible to determine how much of the elevation of blood pressure is neurogenic and how much is fixed.

It is difficult to draw definite conclusions from the small number of observations made in acute glomerulonephritis and in early essential hypertension. The decrease in blood flow found in these cases, however, is evidence in favor of the view that an increase in fixed resistance is the primary process in these diseases. Hypertrophy of the heart may lag behind and eventually cause a further rise in blood pressure and a restoration of the blood flow to within normal limits in the more advanced stages of essential or renal hypertension. In the malignant phase, the hypertrophied heart can probably no longer keep pace with the further increase in resistance by equivalent elevation of the blood pressure, and the blood flow again decreases. It is apparent, therefore, that only in established essential or renal hypertension is the blood pressure an adequate index of the peripheral resistance. In the acute or malignant phase, the actual resistance may be considerably greater than is indicated by the blood pressure level. It should also be noted that the increase in fixed resistance in acute glomerulonephritis is reversible.

From Table III, it is apparent that a decreased digital blood flow is a consistent finding in the first six weeks after a coronary occlusion. This phenomenon was observed in several cases in which the electrocardiograms were equivocal, although the clinical course was characteristic of coronary occlusion. In the doubtful cases, in which both the clinical course was atypical and the electrocardiograms inconclusive, it is probable that the demonstration of a decreased digital blood flow indicated the presence of an acute myocardial infarct.

Since it is possible, on the other hand, for the blood flow to be within normal limits in an occasional case of acute coronary occlusion, the demonstration of a normal digital blood flow cannot be considered absolute evidence of the absence of acute myocardial infarction. In addition, a decrease in digital blood flow is demonstrable only

in the first six weeks, or perhaps in some cases, a few months after the onset of the acute occlusion; for in cases studied several months or more after the acute coronary thrombosis, the digital blood flow was within normal limits. It should also be pointed out that a decrease in digital blood flow is not pathognomonic of coronary occlusion. Such decreases occur in acute and malignant hypertension, in various peripheral vascular diseases and also in systemic conditions associated with shock. Studies of the digital circulation alone in such cases would be difficult to interpret, although they might render valuable information, especially if concomitant measurements of blood volume and other aspects of the circulation were made. The demonstration of decreased digital blood flow can be helpful, however, in the differentiation of a genuine case of coronary thrombosis from angina pectoris.

The correlation between the digital vascular resistance as measured after acute coronary occlusion and the antecedent blood pressure level makes it possible to determine the presence or absence of preëxisting hypertension. The determination of the digital vascular resistance thus offers a method of measuring the extent of the fundamental vascular abnormality in hypertension, even when complicating factors have reduced the blood pressure.

From these studies, the probable mechanism of the circulatory changes produced by an acute coronary occlusion may be visualized as follows: A decrease in myocardial contractile force is produced by the infarction. This operates against an unchanged vascular resistance or a resistance increased by compensatory vasoconstriction to bring about a decrease in arterial blood pressure. The decrease in arterial blood pressure is not, in other words, caused by a decrease in resistance, but largely by a decrease in the cardiac driving force. The decrease in arterial blood pressure, together with such compensatory vasoconstriction as takes place, causes a decrease in systemic blood flow, venous return, and cardiac output. This produces a further decrease in blood pressure. The process, therefore, is probably one of progressive deceleration of the circulation, ultimately balanced at a lower equilibrium by those forces which tend to keep the blood flow normal.

SUMMARY AND CONCLUSIONS

1. Methods for calculating the digital blood flow from calorimetric observations have been modified and a method developed for calculating digital vascular resistance.

2. After vasodilatation produced by warming the body, normal digital blood pressure, blood flow, and vascular resistance were found in patients with neurogenic elevations of blood pressure. A consistent increase in digital vascular resistance and a normal digital blood flow were demonstrated in established essential or renal hypertension. In acute and in malignant hypertension, the digital blood flow may be decreased, and the vascular resistance increased out of proportion to the elevation in blood pressure.

3. Decrease in digital blood flow and blood pressure, and unchanged vascular resistance were demonstrated in cases of acute coronary occlusion, whether the antecedent blood pressure was normal or elevated.

This opportunity is taken of acknowledging the cooperation of Drs. B. S. Oppenheimer, G. Baehr, E. Moschowitz, and E. P. Boas in making these cases available for study, and of Dr. A. M. Master, in whose department some of these studies were carried out.

BIBLIOGRAPHY

1. Mendlowitz, M., Measurements of blood flow and blood pressure in clubbed fingers. *J. Clin. Invest.*, 1941, 20, 113.
2. Mendlowitz, M., Schauer, G., and Gross L., Hemodynamic studies in experimental coronary occlusion. III. Denervated heart experiments. *Am. Heart J.*, 1937, 14, 21.
3. Stewart, G. N., Blood flow in the hand, normal and pathological. *Harvey Lectures*, 1912, 8, 86.
4. Böger, A., and Wezler, K., Die Bestimmung des arteriellen Gesamtwiderstandes am Menschen. *Arch. f. exper. Path. u. Pharmacol.*, 1937, 186, 43.
5. Bazett, H. C., Cotton, F. S., Laplace, L. B., and Scott, J. C., The calculation of cardiac output and effective peripheral resistance from blood pressure measurements with an appendix on the size of the aorta in man. *Am. J. Physiol.*, 1935, 113, 312.
6. Whittaker, S. R. F., and Winton, F. R., The apparent viscosity of blood flowing in the isolated hind limb of the dog and its variation with corpuscular concentration. *J. Physiol.*, 1933, 78, 339.
7. Gaertner, G., Über einen neuen Blutdruckmesser (Tonometer). *Wien. klin. Wchnschr.*, 1899, 12, 696.

8. Von Recklinghausen, H., Unblutige Blutdruckmessung. Arch f. exper. Path. u. Pharmacol., 1906, 55, 375.
9. Fishberg, A. M., Heart Failure. Lea and Febiger, Philadelphia, 1940, 2nd edition, p. 456.
10. Fick, A., Ueber die Messung des Blutquantums in den Herzventrikeln. Verh. d. Physikalisch-Medizinischen Gesellschaft zu Wurzburg, 1870, 2, 16.
11. Grollman, A., The Cardiac Output of Man in Health and Disease. Charles C. Thomas, Springfield, Ill., 1932.
12. Starr, I., Rawson, A. J., Schroeder, H. A., and Joseph, N. R., Studies on estimation of cardiac output in man, and of abnormalities in cardiac function, from heart's recoil and blood's impacts; ballistocardiogram. Am. J. Physiol., 1939, 127, 1.
13. Wiggers, C. J., Basic hemodynamic principles essential to interpretation of cardiovascular disorders. Bull. N. Y. Acad. Med., 1942, 18, 3.
14. Prinzmetal, M., and Wilson, C., The nature of the peripheral resistance in arterial hypertension with special reference to the vasomotor system. J. Clin. Invest., 1936, 15, 63.
15. Grant, R. T., Observations on blood circulation in voluntary muscle in man. Clin. Sc., 1938, 3, 157.
16. Abramson, D. I., Resting peripheral blood flow in hypertensive subjects. Proc. Soc. Exper. Biol. and Med., 1940, 45, 127.
17. Wilkins, R. W., and Eichna, L. W., Blood flow to the forearm and calf. Bull. Johns Hopkins Hosp., 1941, 68, 425.
18. Stead, E. J., Jr., and Kunkel, P., The nature of peripheral resistance in arterial hypertension. J. Clin. Invest., 1940, 19, 25.
19. Hewlett, A. W., and van Zwaluwenburg, J. G., The rate of blood flow in the arm. Heart, 1909, 1, 87.
20. Wilkins, R. W., Doupe, J., and Newman, H. W., The rate of blood flow in normal fingers. Clin. Sc., 1938, 3, 403.
21. Pickering, G. W., The peripheral resistance in persistent arterial hypertension. Clin. Sc., 1936, 2, 209.
22. Harris, K. E., and Marvin, H. M., A note on the temperature of venous blood and its use in estimating rate of blood flow to the hand. Heart, 1927, 14, 49.
23. Mendlowitz, M., Clubbed fingers and hypertrophic osteoarthropathy. Medicine, 1942. (In press.)

THE DIGITAL CIRCULATION IN PERIPHERAL VASCULAR DISEASES

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In a previous communication (1), methods were described for studying the digital blood flow, blood pressure, and vascular resistance in normal subjects and in patients with hypertension and coronary thrombosis. These methods have been applied to the investigation of Raynaud's syndrome, scleroderma, and thrombo-angiitis obliterans involving the upper extremities.

RESULTS

The results are presented in Table I. In three cases of typical Raynaud's disease, one of less than one year's duration, and the others of less than

extreme decrease in digital blood flow to approximately four per cent of the normal, with a correspondingly extreme increase in digital vascular resistance to the height of 1740 units (normal 63 to 96 units). The digital arterial blood pressure was somewhat low and the brachial-digital arterial pressure gradient moderately increased.

Seven cases of scleroderma were studied. In one case (C. R.) only the neck, chest, face, and legs were involved, there being no perceptible sclerodactyly. In this case, the digital blood flow was at the lower limit and the digital vascular resistance at the upper limit of normal. In the

TABLE I
Peripheral vascular diseases

Patient	Age and sex	Diagnosis	Duration	After body warming			
				Brachial blood pressure	Digital blood pressure	Digital blood flow	Digital vascular resistance
				mm. Hg	mm. Hg	cc. per sq. cm. per minute	units
R. N.	52 F	Raynaud's disease	2 years	128/75	110/66	0.22	112
P. B.	23 F	Raynaud's disease	2 years	112/78	110/65	0.20	123
R. B.	46 F	Raynaud's disease	Less than 1 year	122/82	110/68	0.19	132
F. S.	45 M	Raynaud's syndrome; secondary sclerodactyly	7 years	116/84	74/54?	0.01	1740
C. R.	39 F	Scleroderma; no sclerodactyly	?	102/72	96/68	0.23	99
J. S.	49 F	Scleroderma; essential hypertension	?	198/118	182/90	0.14	279
F. U.	37 F	Scleroderma	6 months	126/88	108/62	0.19	126
L. E.	51 F	Scleroderma	9 years?	125/65	110/45	0.14	153
D. C.	43 F	Scleroderma	5 years	94/58	68/55	0.09	186
H. T.	56 M	Scleroderma	5 years	120/90	90/65	0.02	1080
E. S.	48 F	Scleroderma	13 years	112/74	58/48	0.03	470
I. S.	33 M	Thrombo-angiitis obliterans	3 years	120/82	35/25?	0.13	54
W. D.	32 M	Thrombo-angiitis obliterans	3 years	128/88	65/54	0.15	105

two, the digital blood flow was below the lower limit of normal, and the digital vascular resistance definitely elevated in each case. The digital blood pressures and the brachial-digital pressure gradients, on the other hand, were normal. In a case of Raynaud's syndrome of seven years' duration in a male, with secondary sclerodactyly and incipient necrosis of the finger tips, there was an

other six cases, in which the scleroderma was accompanied by sclerodactyly, there was a uniform decrease in digital blood flow and an increase in digital vascular resistance. The extent of these changes was roughly proportional to the duration of the disease. In the two most advanced cases, there was also a moderate decrease in digital arterial pressure and a corresponding increase in

the brachial-digital arterial pressure gradient. In one of the cases of scleroderma and sclerodactyly, there was associated essential hypertension which was not in the acute or malignant stage. The digital blood flow in this case, however, was decreased, and the vascular resistance was elevated beyond the level commensurate with the degree of hypertension (1).

In two cases of thrombo-angiitis obliterans with upper extremity involvement, the digital arterial blood pressure was very low in both. There was a corresponding pronounced increase in the brachial-digital arterial pressure gradient. The digital blood flow in each case was decreased in proportion to the decrease in digital arterial pressure, the digital vascular resistance being comparatively normal. There were no changes in digital vascular resistance in any way comparable to those found in Raynaud's disease or scleroderma.

DISCUSSION

In these investigations, the circulation in the finger tip was studied after release of sympathetic tone by immersion of the contralateral upper extremity in a water bath, kept between forty-three and forty-five degrees Centigrade, and stirred until generalized sweating was well advanced. At this stage of vasodilatation Lewis and Pickering (2) were unable to effect any significant increase in the temperature of the fifth finger by novocaine injection of the ulnar nerve. The effect of body warming in releasing sympathetic vasoconstrictor nerve tone has also been shown to be operative in cases of Raynaud's disease and scleroderma (3, 4). Our results, therefore, represent the fixed irreversible changes in the digital circulation unaffected by fluctuations in vasoconstrictor nerve tone.

The decreased blood flow and increased vascular resistance in early Raynaud's disease indicates that some organic obstruction of the small blood vessels may occur very early in the course of the disease. The acute attacks, then, are produced by functional reversible vasoconstriction, superimposed upon whatever organic obstruction is present. In the more advanced stages of Raynaud's disease, secondary sclerodactyly contributes to the further organic constriction of the vascular bed.

In scleroderma, there may be little if any change in the digital circulation in the absence of sclerodactyly. In most cases, however, there is a decrease in digital blood flow and an increase in resistance very early in the course of the disease. In fact, the diagnosis may be established in doubtful cases by demonstrating these changes in the digital circulation. In the very advanced stages, the tight skin may compress the main digital arteries themselves (5, 6) and thus produce some decrease in digital arterial pressure and therefore an increased brachial-digital arterial pressure gradient. It is impossible to determine from these investigations to what extent the obstruction of the blood vessels in scleroderma is intravascular or extravascular.

In thrombo-angiitis obliterans, the decrease in digital blood flow was attributable entirely to the decrease in digital arterial pressure, the resistance of the smaller blood vessels being unaffected. The extreme increase in the brachial-digital arterial pressure gradient in these cases was probably caused by vascular obstruction confined to the larger arteries.

SUMMARY AND CONCLUSIONS

1. The digital circulation was studied in Raynaud's syndrome, scleroderma, and thrombo-angiitis obliterans.
2. In Raynaud's disease and in scleroderma, the digital blood flow is usually decreased and the digital vascular resistance increased. In thrombo-angiitis obliterans, the digital blood flow is decreased because of a decrease in digital arterial blood pressure, the digital vascular resistance remaining comparatively unchanged.
3. Study of the digital circulation may be useful as an aid in diagnosis and prognosis, and as an index of the effect of therapy in peripheral vascular diseases involving the upper extremities.

This opportunity is taken of acknowledging the co-operation of Drs. G. Baehr, E. Moschowitz, E. P. Boas, and S. Silbert in making these cases available for study.

BIBLIOGRAPHY

1. Mendlowitz, M., The digital blood flow, arterial pressure and vascular resistance in arterial hypertension and in coronary thrombosis. *J. Clin. Invest.*, 1942, 21, 539.

2. Lewis, T., and Pickering, G. W., Vasodilatation in the limbs in response to warming the body; with evidence for sympathetic vasodilator nerves in man. *Heart*, 1931, 16, 33.
3. Lewis, T., Experiments relating to the peripheral mechanism involved in spasmodic arrest of the circulation in the fingers, a variety of Raynaud's disease. *Heart*, 1929, 15, 7.
4. Lewis, T., and Landis, E. M., Further observations upon a variety of Raynaud's disease; with special reference to arteriolar defects and to scleroderma. *Heart*, 1931, 15, 329.
5. Prinzmetal, M., Studies of the mechanism of circulatory insufficiency in Raynaud's disease in association with sclerodactylia. *Arch. Int. Med.*, 1936, 58, 309.
6. Sodeman, W. A., and Burch, G. E., A direct method for the estimation of skin distensibility with its application to the study of vascular states. *J. Clin. Invest.*, 1938, 17, 785.

THE MEASUREMENT AND RECORDING OF GASTRODUODENAL BLOOD FLOW IN MAN BY MEANS OF A THERMAL GRADIENTOMETER¹

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(Received for publication March 16, 1942)

Measurement of blood flow in the intact human gut is desirable, not only because of its significance in physiological and pharmacological problems, but also because circulatory changes may be implicated in the mechanism of peptic ulceration. Studies on intestinal blood flow involving extensive surgical procedures with cannulation of arteries and veins have been carried out on animals (1 to 6), but the observations for the most part are contradictory, and, at best, deductions derived from them cannot be applied directly to human conditions. In other studies, inferences about the intestinal blood flow have been drawn from direct inspection of changes in calibre of the serosal vessels (7) and changes in color of the mucosa (8, 9).

METHOD PREVIOUSLY DESCRIBED

Recently, we reported a method of measuring and recording mucosal blood flow in man. This required of the subject only that he swallow a duodenal tube with a small balloon attached (10). On the surface of this balloon was mounted the measuring element consisting of a silver button with a heating coil and a thermocouple. The button was applied to the mucosa by inflating the balloon with air to a pressure of about 15 to 20 mm. Hg, and the reference couple was maintained at body temperature. When in place, the button was heated about 2° C. above body temperature by connecting the heating coil to a source of constant current. Under these conditions, the actual temperature of the button varied according to the amount of heat lost by conduction to the blood flowing past it.

Two possible sources of error in the use of this apparatus were: (1) A change in area of the button actually in contact with the mucosa; and (2) movement of the button to a new position on the mucosa. In the first instance, the area of contact would affect the temperature

of the button, and no method was available to distinguish between this effect and a change in blood flow. The second possibility of error was due to the fact that the temperature of the button was above that of the body and hence the tissue directly beneath it was warmer than the surrounding area. Therefore, any shift to an unheated area would cool the button until equilibrium was reestablished. This effect was impossible to distinguish from a change in contact area or a change in blood flow.

PRESENT METHOD

After trying several methods of circumventing these difficulties, the silver button was finally discarded in favor of the arrangement here described (Figure 1). A balloon, about 8 cm. in length and 2.5 cm. in its greatest diameter, has moulded into its wall 6 constantan-copper thermocouples equally spaced around its greatest circumference. The duodenal tube passes through the center of the balloon and on this tube is wound the heating coil with the reference thermocouples beneath it. The temperature of the heater is raised about 10° C. above that of the body and heat is radiated to the whole surface of the balloon. Consequently, all the tissue in contact with it is warmed slightly above body temperature.

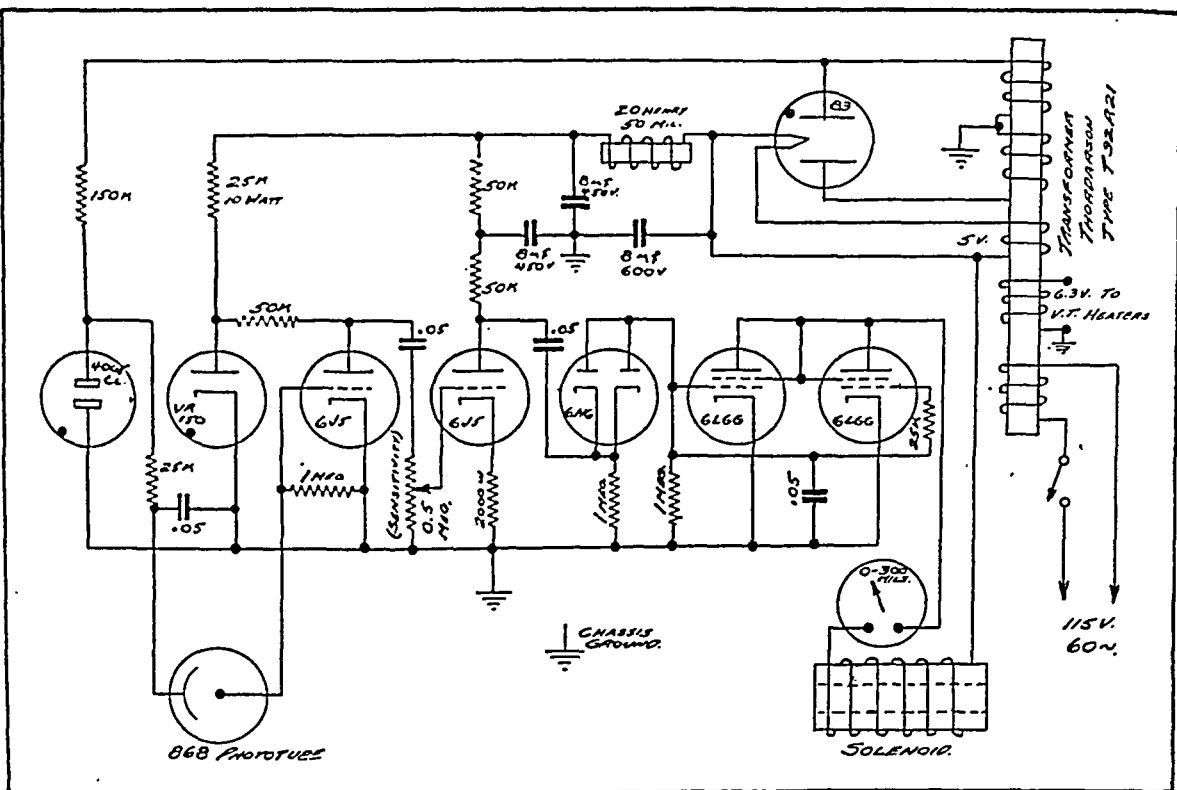
With this instrument, the errors previously encountered are minimized: (1) Since measurement is made at 6 points on the surface of the balloon and the couples are in series, the average temperature gradient between the heater and the balloon wall is recorded and the area of contact is less important; (2) since a much greater area of tissue is warmed than is actually used, considerable movement along the mucosa is possible without disturbing equilibrium at the points from which measurement is being made.

The apparatus was used with the subject in a fasting state to minimize the cooling effect which might be produced by the flow of intestinal contents past the balloon. To determine whether this factor could account for any of the observed results, a special balloon was constructed which was provided with a by-pass. Since experiments with this balloon gave identical results, it was assumed that this possible source of error was negligible.

It is important to note that this device measures the flow of blood past the balloon and not the amount of blood in the tissues. A decreased flow would be registered from a mucosa engorged with blood, if stasis and congestion were present.

¹ This investigation has been aided by a grant from the Josiah Macy, Jr. Foundation.

² National Research Council Fellow in the Medical Sciences.



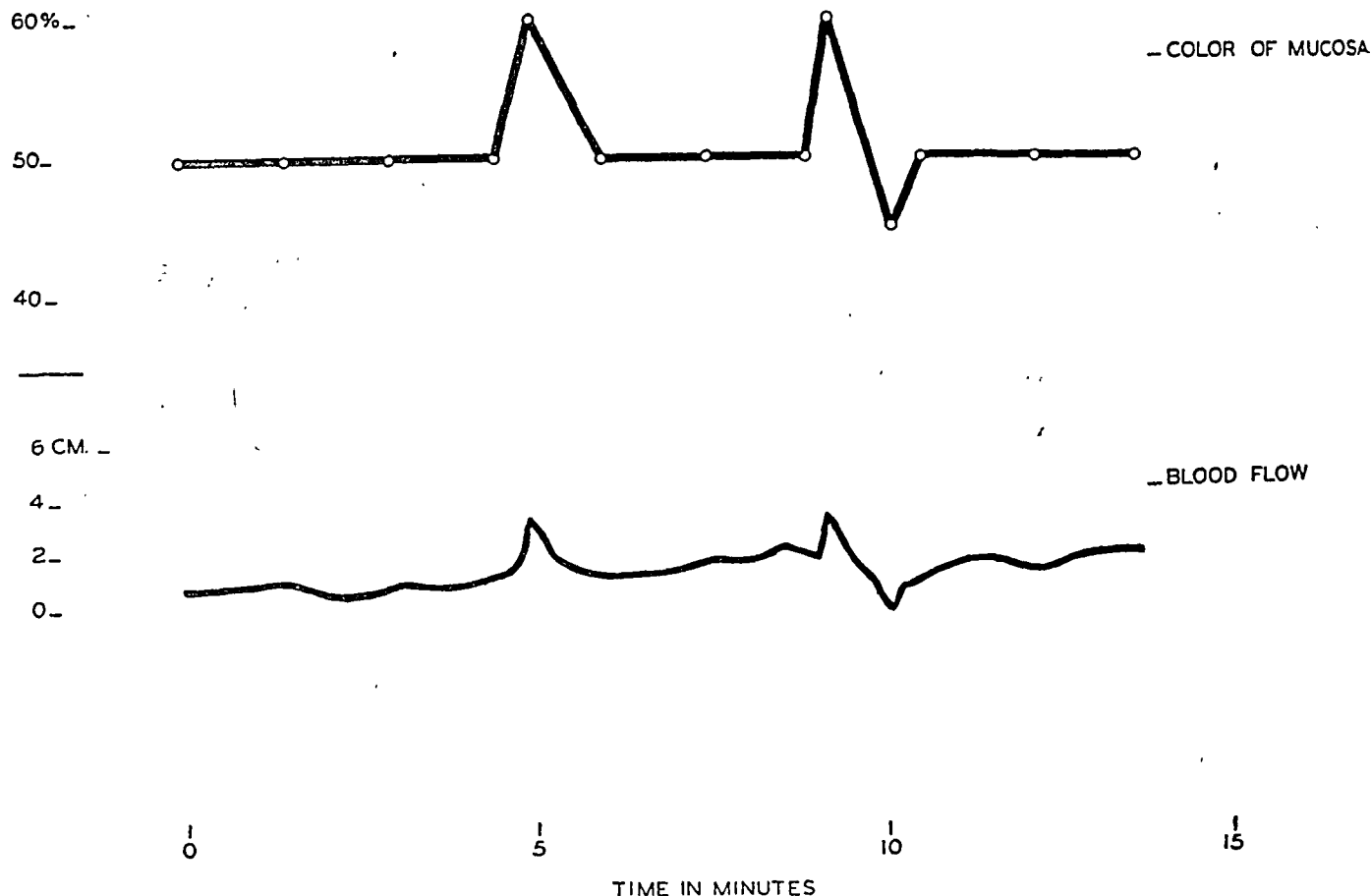


FIG. 4. SPONTANEOUS CHANGES IN RECORDED BLOOD FLOW IN THE STOMACH CORRELATED WITH CHANGES IN THE COLOR OF THE MUCOUS MEMBRANE NOTED ON DIRECT INSPECTION

The scale on the ordinate shows in centimeters the actual excursion of the recording needle from the base line with increase in blood flow.

in recorded blood flow following histamine paralleled the color changes of the gastric mucosa as ascertained by direct observation.

4. *Effect of amyl nitrite and nitroglycerin on blood flow.* Amyl nitrite inhalation (1 cc.) and 0.6 mgm. of nitroglycerin placed under the tongue were followed by a variety of circulatory changes. Usually within 1 to 2 minutes after administration of these agents the gastric mucosa had become more red and engorged. At such times a simultaneous increase in blood flow occurred. At other times, especially with larger amounts of the nitrites and a fall in blood pressure, the mucosa became slightly cyanotic and the recorded blood flow either remained unchanged or was diminished.

OBSERVATIONS OF THE DUODENUM

1. *Effect of motility on blood flow.* As in the stomach, each short contraction of the duodenum was accompanied by a transitory acceleration of recorded blood flow. This effect is illustrated

in Figure 6. During phases of frequent contractions, the base line is significantly higher than during quiescent phases.

2. *Response to appetizing stimuli.* The sight and smell of food in a hungry patient produced the effect on blood flow and motility which is shown in Figure 7. With a steady base line in both tracings, food was presented at the arrow. Within less than a half a minute strong contractions started and within one and a half minutes the blood flow began to increase. The latter effect lasted about 6 minutes, although the motility was altered for a longer period. Food which was not appetizing, on the other hand, evoked no such effects. Indeed, when actual distaste was encountered, a decrease in blood flow occurred. Figure 8 shows a sharp increase in blood flow and motility which occurred following a mere discussion of appetizing foods.

3. *Changes associated with anxiety, tension and resentment.* Feelings of anxiety, tension and re-

sentiment were evoked in one subject during an interview which focussed upon experiences with a business partner, who had in the subject's opinion betrayed and deceived him. A detailed per-

sonality study of this patient is presented elsewhere (11). In Figure 9, an increase in blood flow is shown to be associated with this distressing emotional state. This graph was made by plot-

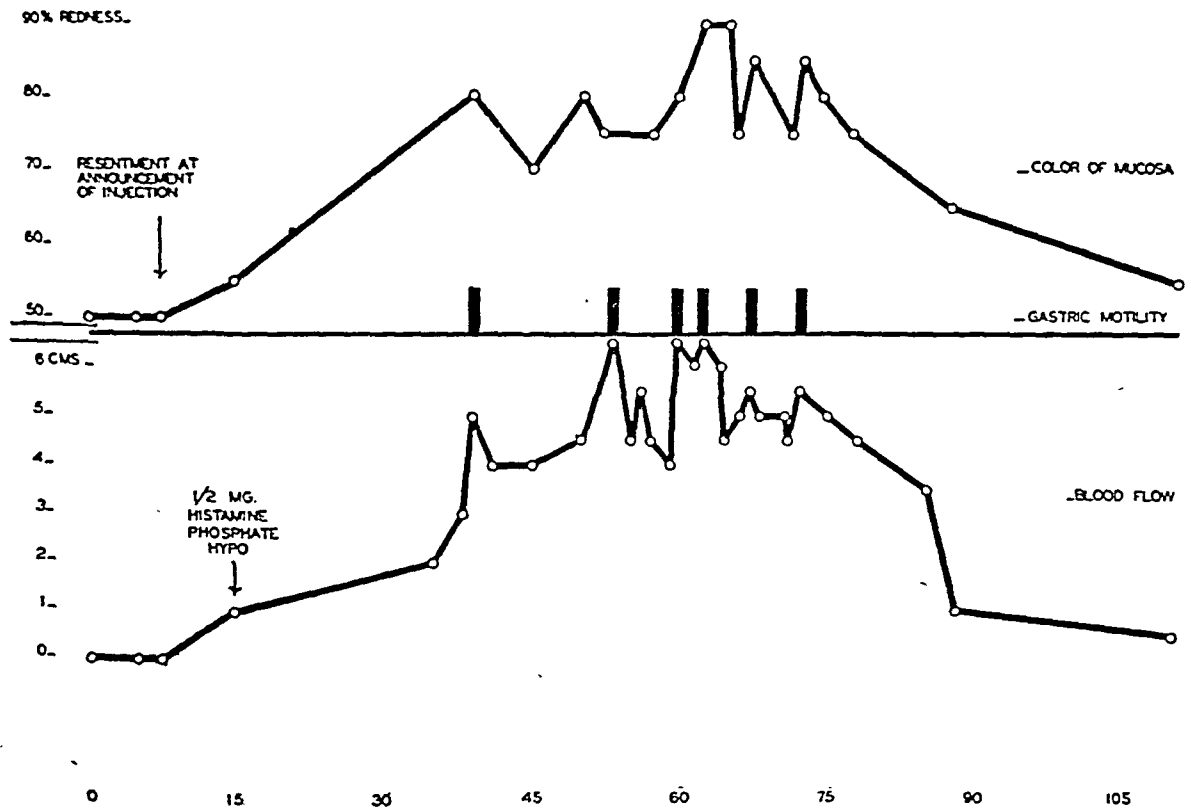


FIG. 5. CORRELATION OF COLOR CHANGES AND CHANGES IN RECORDED BLOOD FLOW IN THE GASTRIC MUCOSA AFTER HISTAMINE

Note transitory blushing and increased blood flow accompanying each major contraction. The contractions are indicated diagrammatically as solid columns. The record of blood flow is represented diagrammatically. The scale on the ordinate shows in centimeters the actual excursion of the recording needle from the base line with increase in blood flow.

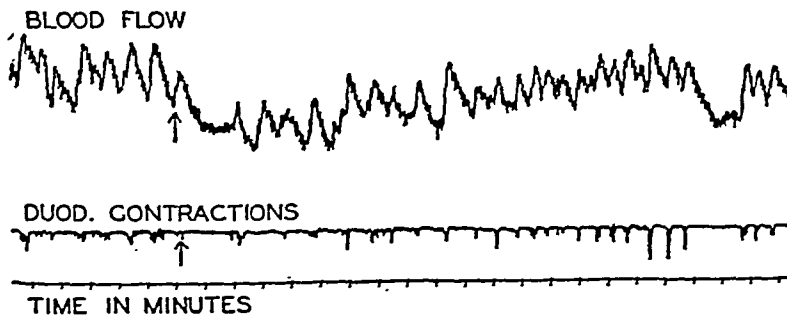


FIG. 6. CORRELATION OF BLOOD FLOW WITH CONTRACTIONS OF THE WALL OF THE DUODENUM

The tracings of blood flow and motility are synchronous.

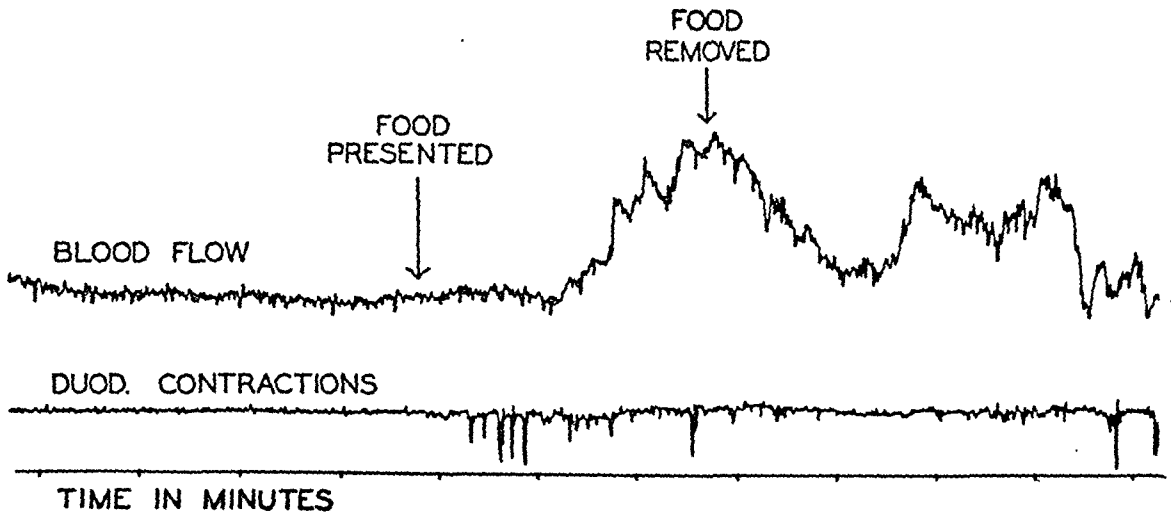


FIG. 7. CHANGES IN BLOOD FLOW AND MOTILITY ASSOCIATED WITH THE SIGHT AND SMELL OF APPETIZING FOOD

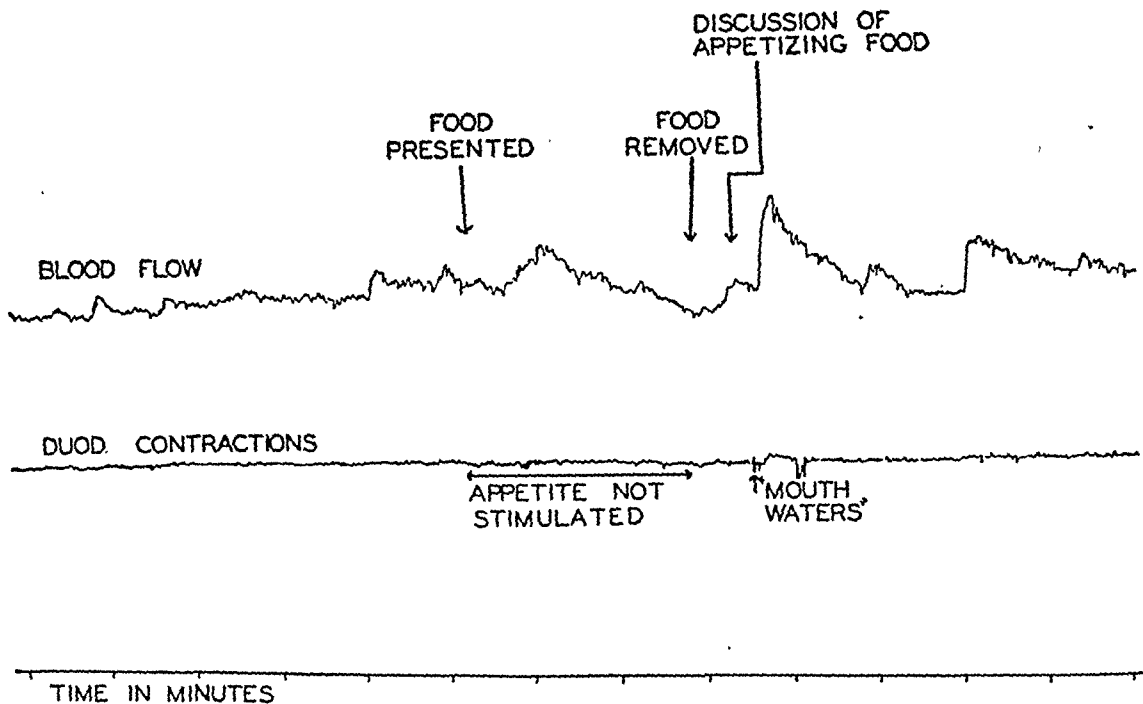


FIG. 8. CHANGES IN BLOOD FLOW AND MOTILITY ASSOCIATED WITH THE DISCUSSION OF APPETIZING FOOD

Note the absence of changes with the sight and smell of unappetizing food.

ting readings obtained directly from the galvanometer and manometer at 5 second intervals.

DISCUSSION

Changes in blood flow in the gastric and duodenal mucosae are here shown to occur under particular circumstances.

The transient increase in blood flow accompanying contractions of the stomach or duodenum is

probably due to the mechanical effect of squeezing the blood out of the muscularis into the mucosa. If the contractions occur frequently enough, they may cause a sustained increase in flow. However, this factor alone cannot account for the large changes in flow shown in Figures 5, 7, 8, 9. It is probable for example that the increase with histamine is due to increased metabolic activity associated with hypersecretion, and the effect of the

ADRENAL CORTICAL HYPERPLASIA WITH VIRILISM: DIAGNOSIS, COURSE AND TREATMENT^{1,2}

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(Received for publication April 7, 1942)

INTRODUCTION

The clinical differentiation of patients with adrenal cortical hyperplasia and adrenal cortical carcinoma is often difficult because both of these lesions may give rise to a symptom complex (1 to 4) known as adrenal cortical virilism or as the adrenogenital syndrome.³ The characteristics of the syndrome usually are accelerated growth and skeletal maturation in children, amenorrhea and lack of breast development in girls, and excessive development of masculine secondary sex characteristics, acne, hirsutism, and increased musculature in individuals of both sexes. Rarely, patients with carcinoma of the adrenal cortex show abnormal feminization instead of masculinization.

The possibility of differentiating hyperplasia from carcinoma by chemical measurements was suggested by investigators who isolated and identified certain steroids in unusual quantities from the urine of patients with adrenal cortical carcinoma (5 to 10). The isolation and identification of urinary steroids, however, requires special facilities which are not available in most clinical laboratories. On the other hand, relatively simple procedures are available for the fractionation and colorimetric assay of some of the urinary steroidal constituents (11). Preliminary observations have been reported from this laboratory on the clinical usefulness of urinary steroid assay in differential diagnosis (12).

If the diagnosis is carcinoma of the adrenal cortex, surgical removal of the tumor is clearly the treatment of choice. However, surgical removal of a significant amount of adrenal tissue does not

appear to be of permanent benefit to patients with adrenal cortical hyperplasia (1, 3, 12, 13). On the other hand, suggestively beneficial effects have been obtained by the administration of estrogenic hormones to female patients with virilism associated with adrenal cortical hyperplasia. Dorfman, Wilson and Peters (3) noted a temporary fall in the urinary excretion of male hormones (androgens) and a slight development of the breast tissue in one girl who received large quantities of one of the natural estrogens, estrone. Lissner (14) has also reported that a 17-year-old girl was feminized by injections of the synthetic estrogen, diethylstilbestrol.

The present communication confirms and extends our previous observations (12) by reporting measurements of the pregnanediol and of the alpha and beta alcoholic, non-alcoholic, and total 17-ketosteroid excretion per day of 12 patients with adrenal cortical hyperplasia and 3 patients with adrenal cortical carcinoma.⁴ Additional data are cited from the literature. The study also presents some observations on 2 girls and 1 boy with adrenal cortical hyperplasia, before and after the oral administration of diethyl stilbestrol.

MATERIAL AND METHODS

Of the 12 patients listed under the diagnosis of adrenal cortical hyperplasia (Figure 1), 11 were studied in this laboratory⁵; Case 13 was reported by Fraser, Forbes, Albright, Sulkowitch and Reifenstein (15); Case 6 was investigated both in our laboratory (case 6b) and by Fraser *et al.* (case 6a). All presented the characteristic evidences of adrenal cortical virilism. In 7 of this group, the diagnosis was confirmed by surgical exploration; in

¹ Read before the American Pediatric Society, Skytop, Pennsylvania, May 2, 1942.

² This work was aided by a grant from the Commonwealth Fund of New York.

³ This clinical entity is to be distinguished from Cushing's syndrome which may also be associated with adrenal cortical dysfunction.

⁴ We are greatly indebted to the following physicians for urine samples and case summaries on the following cases reported here: Dr. F. Albright, Cases 13 and 15; Dr. H. Friedgood, Case 16; Dr. R. Ganz, Case 3; Dr. S. Werner, Cases 4, 5 and 12; Dr. G. Twombly, Case 7; Dr. J. Warkany, Case 1; Dr. W. A. Reilly, Case 8.

⁵ Detailed data relating to these patients are on file in this office.

8. Drury, A. N., Florey, H., and Florey, M. E., The vascular reactions of the colonic mucosa of dog to fright. *J. Physiol.*, 1929, 68, 173.
9. Wolf, S., and Wolff, H. G., A Man and His Stomach. (To be published.)
10. Richards, C. H., Wolf, S., and Wolff, H. G., Studies on blood flow in the gastrointestinal tract in man. *J. Clin. Invest.*, 1941, 20, 440.
11. Wolf, S., and Wolff, H. G., Intermittent fever of unknown origin: Study of recurrent high fever with benign outcome in a patient with migraine, and notes on "neurogenic fever." *Arch. Int. Med.*, 1942. (In press.)

ADRENAL CORTICAL HYPERPLASIA WITH VIRILISM: DIAGNOSIS, COURSE AND TREATMENT^{1,2}

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The possibility of differentiating hyperplasia from carcinoma by chemical measurements was suggested by investigators who isolated and identified certain steroids in unusual quantities from the urine of patients with adrenal cortical carcinoma (5 to 10). The isolation and identification of urinary steroids, however, requires special facilities which are not available in most clinical laboratories. On the other hand, relatively simple procedures are available for the fractionation and colorimetric assay of some of the urinary steroidal constituents (11). Preliminary observations have been reported from this laboratory on the clinical usefulness of urinary steroid assay in differential diagnosis (12).

If the diagnosis is carcinoma of the adrenal cortex, surgical removal of the tumor is clearly the treatment of choice. However, surgical removal of a significant amount of adrenal tissue does not

appear to be of permanent benefit to patients with adrenal cortical hyperplasia (1, 3, 12, 13). On the other hand, suggestively beneficial effects have been obtained by the administration of estrogenic hormones to female patients with virilism associated with adrenal cortical hyperplasia. Dorfman, Wilson and Peters (3) noted a temporary fall in the urinary excretion of male hormones (androgens) and a slight development of the breast tissue in one girl who received large quantities of one of the natural estrogens, estrone. Lisser (14) has also reported that a 17-year-old girl was feminized by injections of the synthetic estrogen, diethylstilbestrol.

The present communication confirms and extends our previous observations (12) by reporting measurements of the pregnanediol and of the alpha and beta alcoholic, non-alcoholic, and total 17-ketosteroid excretion per day of 12 patients with adrenal cortical hyperplasia and 3 patients with adrenal cortical carcinoma.⁴ Additional data are cited from the literature. The study also presents some observations on 2 girls and 1 boy with adrenal cortical hyperplasia, before and after the oral administration of diethyl stilbestrol.

MATERIAL AND METHODS

Of the 12 patients listed under the diagnosis of adrenal cortical hyperplasia (Figure 1), 11 were studied in this laboratory⁵; Case 13 was reported by Fraser, Forbes, Albright, Sulkowitch and Reifstein (15); Case 6 was investigated both in our laboratory (case 6b) and by Fraser *et al.* (case 6a). All presented the characteristic evidences of adrenal cortical virilism. In 7 of this group, the diagnosis was confirmed by surgical exploration; in

¹ Read before the American Pediatric Society, Skytop, Pennsylvania, May 2, 1942.

² This work was aided by a grant from the Commonwealth Fund of New York.

³ This clinical entity is to be distinguished from Cushing's syndrome which may also be associated with adrenal cortical dysfunction.

⁴ We are greatly indebted to the following physicians for urine samples and case summaries on the following cases reported here: Dr. F. Albright, Cases 13 and 15; Dr. H. Friedgood, Case 16; Dr. R. Ganz, Case 3; Dr. S. Werner, Cases 4, 5 and 12; Dr. G. Twombly, Case 7; Dr. J. Warkany, Case 1; Dr. W. A. Reilly, Case 8.

⁵ Detailed data relating to these patients are on file in this office.

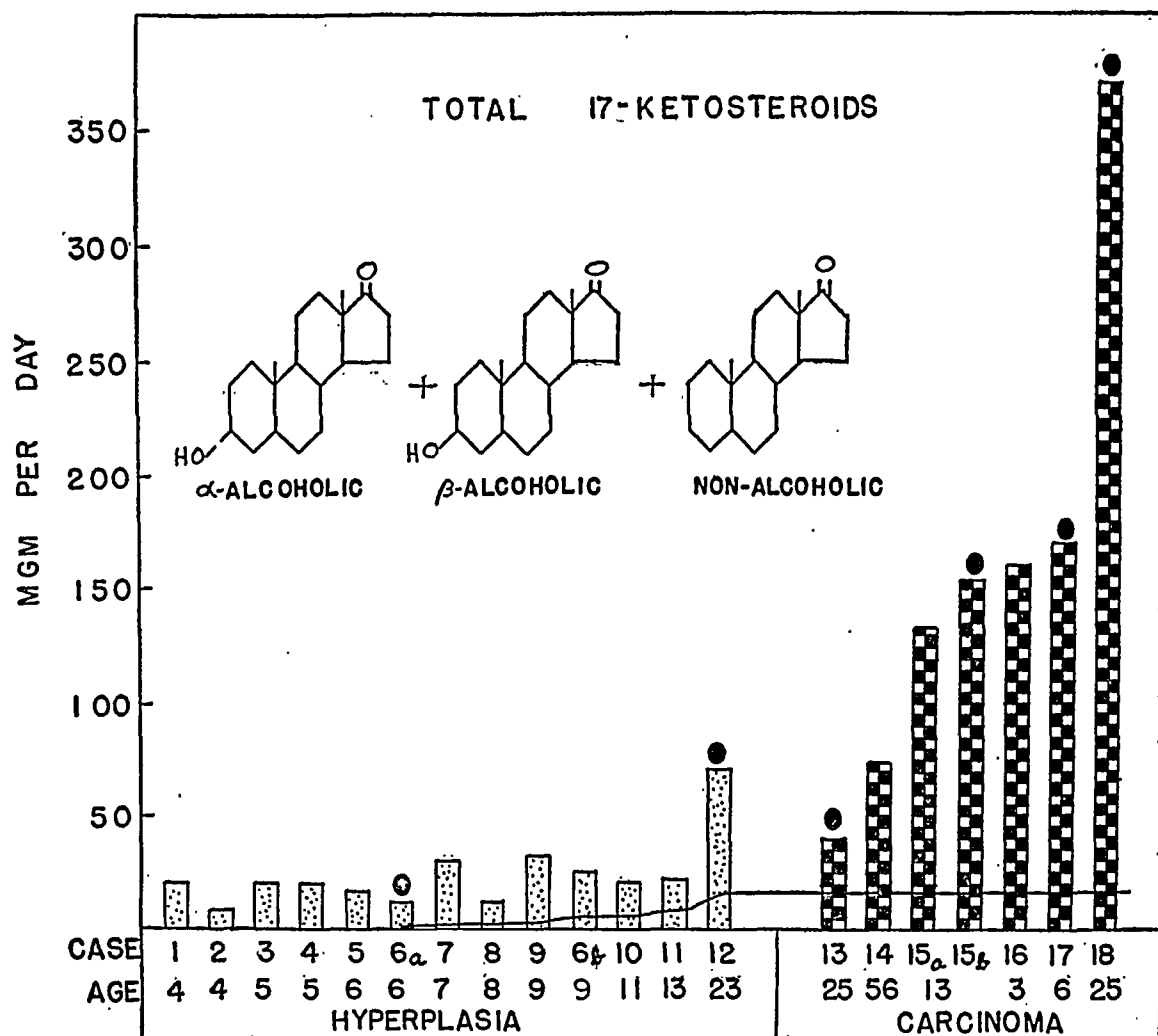


FIG. 1. TOTAL URINARY 17-KETOSTEROID OUTPUT PER DAY BY PATIENTS WITH ADRENAL CORTICAL HYPERPLASIA (SPECKLED COLUMNS) AND WITH ADRENAL CORTICAL CARCINOMA (CHECKERED COLUMNS) AS GIVEN BY THE ORDINATE

A black circle over a column indicates that the measurement was taken from the literature. Cases 6^a and 12, reference 15; cases 13 and 17, reference 5; case 15^b, reference 10; case 18, reference 16; case 19, figure 4, reference 9. The semi-horizontal curve represents the approximate upper limit of normal 17-ketosteroid output per day by normal individuals of the same or of an older age.

the remainder, a presumptive diagnosis was made on the basis of the clinical findings and the prolonged course of the disease.

Three of the 7 patients with adrenal cortical carcinoma (Figures 1 to 4) were studied in this laboratory.⁶ Case 15 was studied both in our laboratory and by Wolfe, Fieser and Friedgood (10). Urinary excretion data on the other 4 were taken from the literature (6, 7, 9, 16). Of these patients, 5 had signs and symptoms of adrenal cortical virilism. On the other hand, Cases 13 and 14 had a different syndrome, commonly known as Cushing's disease, which in these patients was associated with carcinoma of the adrenal cortex.

Estimations of the approximate upper limit of normal

⁶ Detailed data relating to these patients are on file in this office.

urinary total 17-ketosteroid output are derived from over 200 determinations, on 80 normal persons of both sexes ranging in age from a few hours to 45 years. The normal values for the various 17-ketosteroid fractions represent measurements made on 40 individual specimens, and on 7 pooled specimens, each one of which was made up to be representative of a different age group. The maximum normal pregnanediol value is derived from 22 determinations made on males of different ages and on girls who had not reached the menarche.

All determinations in this laboratory were made according to procedures described elsewhere (11, 17). With the exception of Case 18 the urinary excretion data on the carcinoma patients taken from the literature represents values obtained by chemists who isolated and identified the urinary steroids.

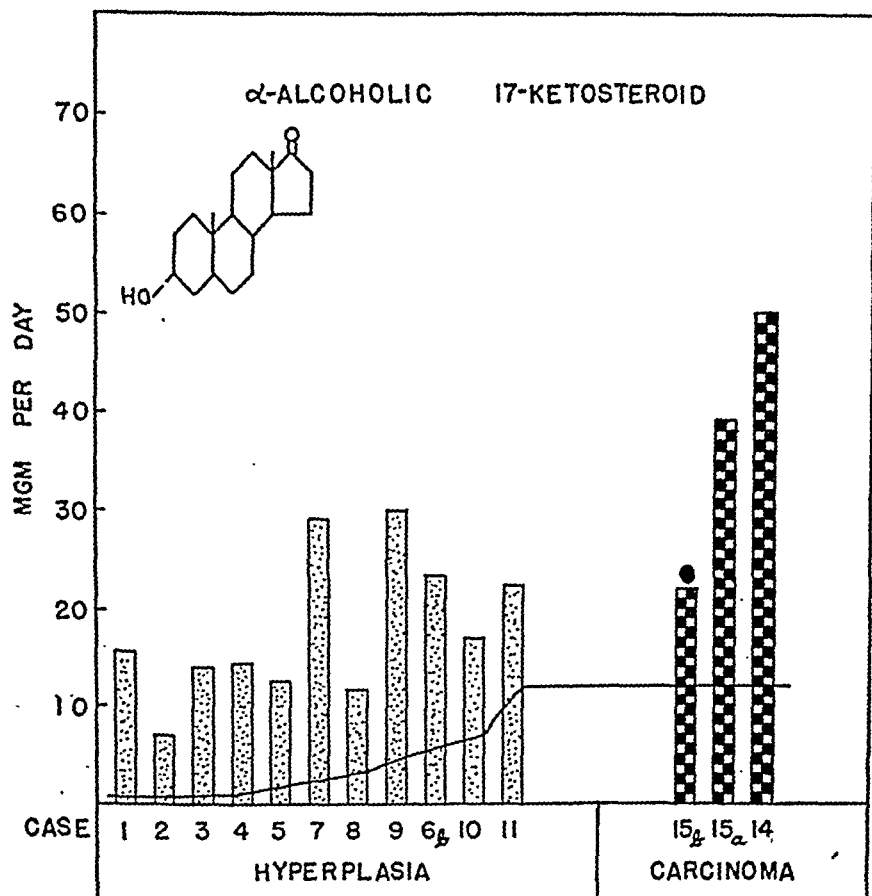


FIG. 2. ALPHA-ALCOHOLIC URINARY 17-KETOSTEROID OUTPUT PER DAY

The various symbols have the same significance as in Figure 1.

RESULTS

A. Daily urine excretion of steroids

Table I gives the data on the urines of abnormal subjects assayed in this laboratory. Figures 1 to 5 present these data and those culled from the literature, together with the approximate upper limit of excretory values for normal subjects of corresponding age. Near the top of each figure is a diagram describing the chemical structure of the substance or substances mentioned in the title of the figure. It will be noted that in Figure 1 the term, total 17-ketosteroids, includes steroids which possess a carbonyl group (ketone) at position 17 (top right hand end of the steroid molecule). The 17-ketosteroids may be divided into alcoholic and non-alcoholic substances. The alcoholic 17-ketosteroids which have an hydroxyl group at position 3 (lower left hand end of the molecule) are further subdivided into alpha- and beta-alcoholic

steroids according to the spatial position of the hydroxyl group, which determines their precipitability by digitonin. The ordinate for each figure gives a scale of the milligrams of the designated steroid excreted per day. On certain of the urines from cancer patients the results are reported in terms of milligrams per liter.⁷ The height of each column in the figures thus indicates the approximate daily output of steroid by the patient whose case number is given along the abscissa. The columns for patients with the diagnosis of adrenal cortical hyperplasia are speckled; those for patients with carcinoma of the adrenal cortex are checkered. A solid black circle over a column indicates that the value is taken from the literature. The curve running upwards in a semi-horizontal direction from left to right, in Figures 1 to 4, describes the approximate upper limit of

⁷ Cases 15 and 19 are so reported.

mal boys excreted not more than 0.5 mgm. per day of apparent pregnanediol,⁸ a portion of which may be interfering chromogens. Four of the patients with adrenal cortical hyperplasia excreted less than this amount of pregnanediol; 5 excreted between 0.5 and 3.5 mgm. per day. The excretion

⁸ Unpublished observations on non-pregnant females, who were having normal menstrual periods, show that these individuals excreted between zero and 10 mgm. of pregnanediol per day, depending upon the phase of the menstrual cycle.

of pregnanediol by adrenal cortical hyperplasia patients thus appears to be variable.

B. Observations on the spontaneous course and on the influence of unilateral adrenalectomy in children with adrenal cortical hyperplasia

Serial measurements of the height and skeletal age⁹ and of the total urinary 17-ketosteroid out-

⁹ The height and skeletal ages were determined by referring to normal standards giving the average age of

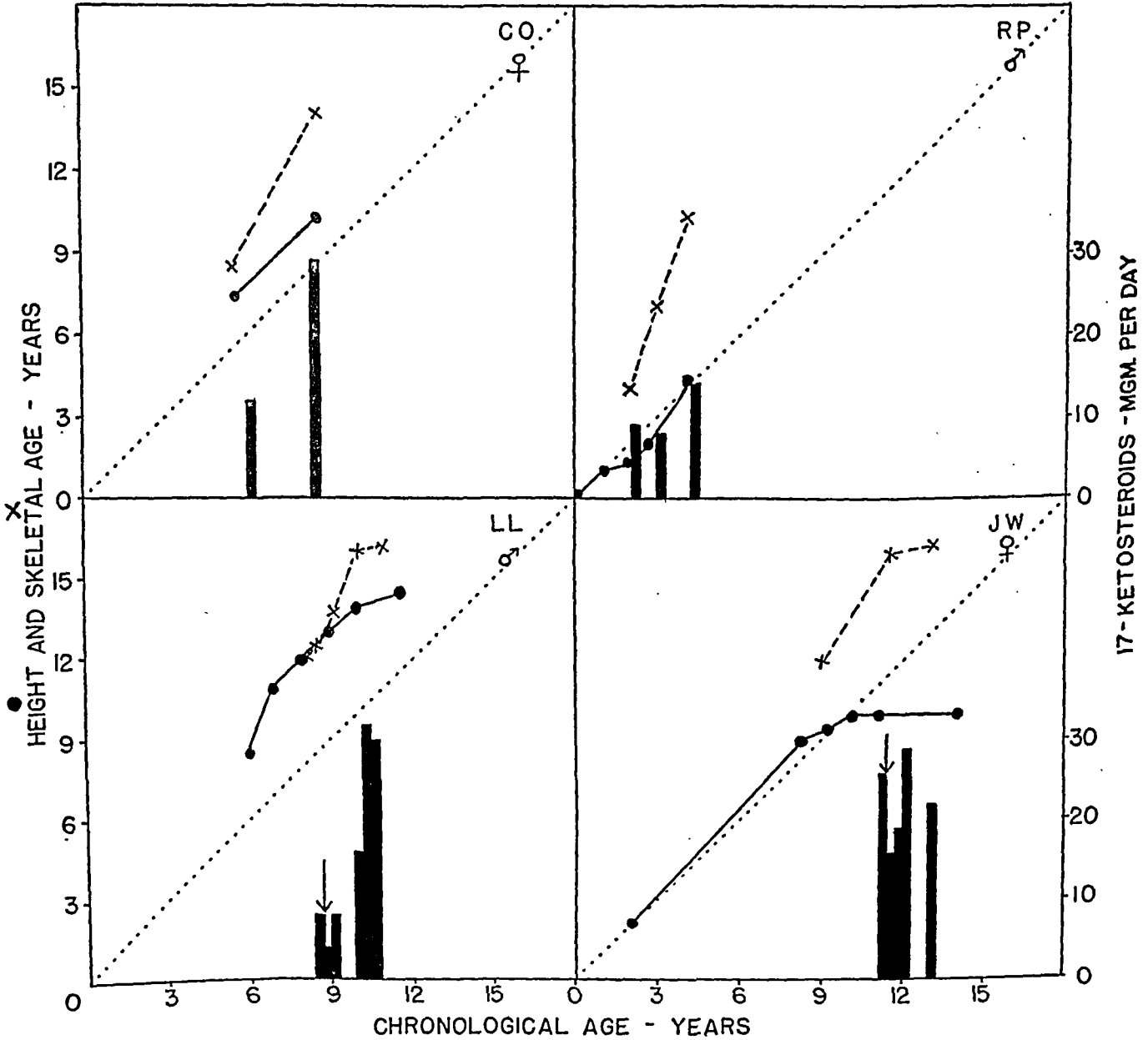


FIG. 6. OBSERVATIONS ON THE HEIGHT AND SKELETAL AGES AND THE TOTAL URINARY 17-KETOSTEROID OUTPUT OF 4 CHILDREN WITH HYPERPLASIA OF THE ADRENAL CORTEX

C. O. is case 6b; R. P. is case 2; L. L. is case 9; and J. W. is case 10. The height age is represented by the black circles, the skeletal age by the crosses, and the 17-ketosteroids by the black columns. The diagonal dotted lines in each square show where the black circles and crosses would fall if the height and skeletal age of the patient corresponded exactly to the chronological age. The arrows in the 2 lower squares indicate that an unilateral adrenalectomy was performed at that time.

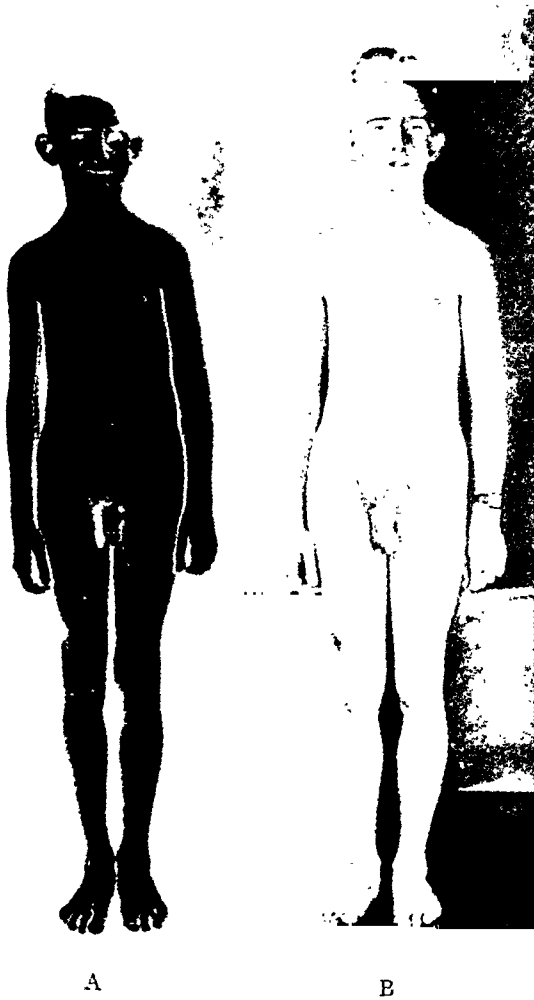


FIG. 7. CASE 9 (L. L.). ADRENAL CORTICAL VIRILISM DUE TO HYPERPLASIA OF THE ADRENAL CORTEX

A. Patient at $7\frac{1}{2}$ years, shortly before unilateral adrenalectomy. B. Patient at $11\frac{1}{2}$ years. Note increase in size of testes.

put per day of 4 children with hyperplasia of the adrenal cortex are plotted as ordinates against their chronological age as abscissae, in Figure 6. The height ages of each patient are represented by black circles connected by solid lines while the skeletal ages are given by crosses connected by broken lines. The 45 degree diagonal dotted lines describe points where the height and skeletal ages would fall if they were exactly equal to their chronological ages. The total 17-ketosteroid output is indicated by the vertical black columns. children of the same sex and height (18) or skeletal development (19).

The arrow in the 2 lower squares indicates that an unilateral adrenalectomy was performed at that time.

The data of Figure 6 indicate that there was a progressive tendency for the skeletal age of these patients to be advanced beyond the chronological age by 3 or more years and for the total urinary 17-ketosteroid output to reach higher levels as the patients grew older. The height age also tended to exceed the chronological age until closure of the bone epiphyses at a skeletal age of approximately 16 years interfered with further growth. The curves show that the operative removal of one adrenal gland in 2 patients (J. W. (Case 10) and L. L. (Case 9)) did not permanently alter the precocious growth and development. Moreover, there was only a temporary drop in the total 17-ketosteroid output. Furthermore, as shown in Figures 7A and B and 8A, B and C, no regression in the masculine secondary sex characters had become evident 2 or more years postoperatively. The left hand photographs (7A, 8A) in these figures were taken just prior to the operation; the photographs designated 7B, 8B and C were made two or more years later. No breast development took place in the girl, and the clitoris of the girl and the penis of the boy failed to diminish in size.

It is noteworthy that the testes of the boy patient (Case 9, Figure 7) had been of normal size for his chronological age from the onset of his symptoms. Thus at approximately 12 years of age, the testes, which had been small in comparison to the abnormally large penis, enlarged, with the result that he then closely resembled a normal adolescent boy in external appearance.

C. Observations on the effect of orally administered diethyl-stilbestrol on patients with adrenal cortical hyperplasia

Some of the physical changes observed in the girl patients, J. W. (Case 10) and C. O. (Case 6), within 6 months after they had started to take 5 to 10 mgm. of diethyl-stilbestrol by mouth daily, are depicted in Figures 8D and 9B. There was a definite and marked increase in mammary gland tissue and in the size of the nipples. The nipples of the latter patient became deeply pigmented. The clitoris of each patient shrank in

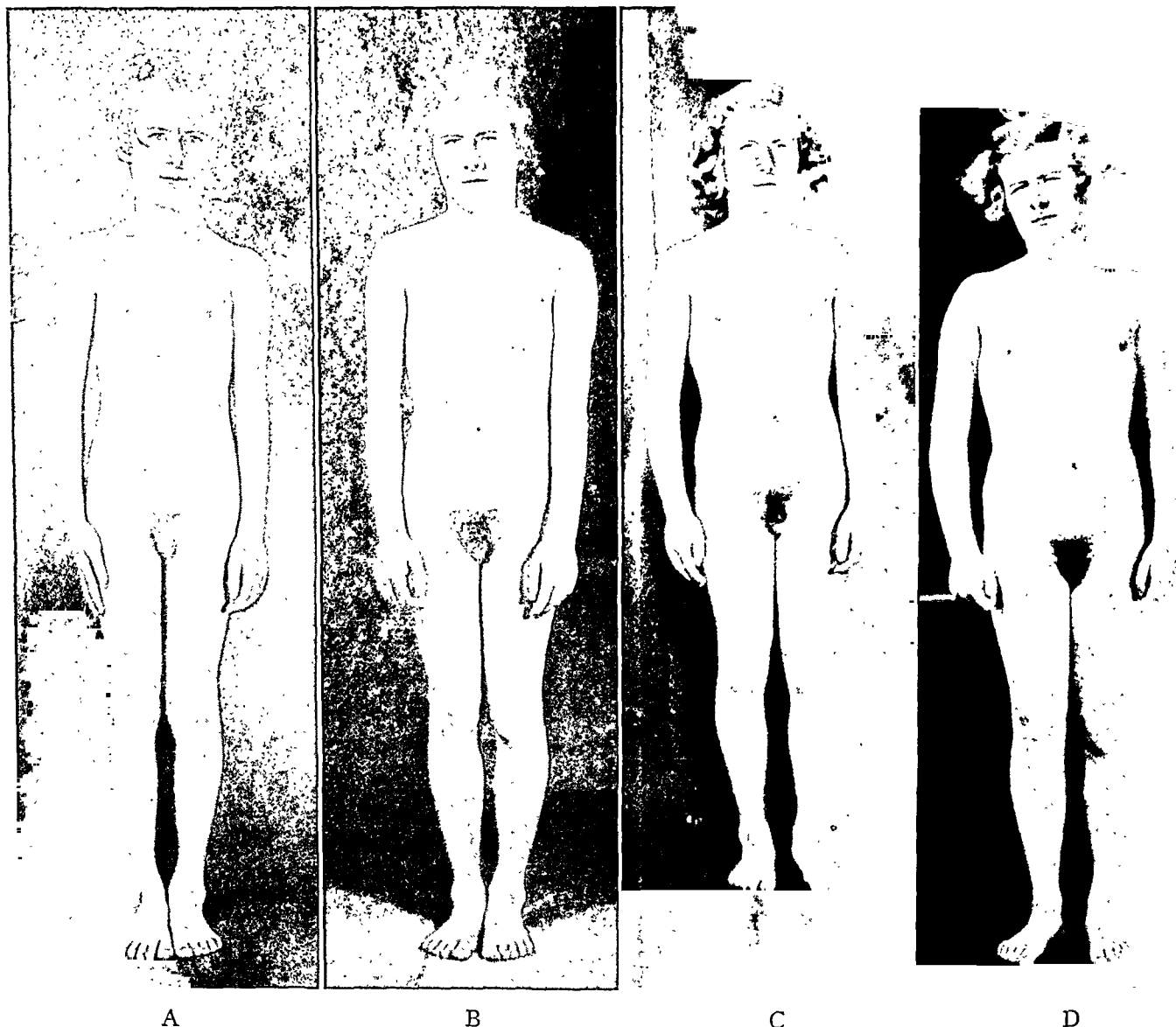


FIG. 8. CASE 10 (J. W.). ADRENAL CORTICAL VIRILISM DUE TO HYPERPLASIA OF THE ADRENAL CORTX

A. Patient at 8 years, shortly before unilateral adrenalectomy. B. Patient at 11½ years. C. Patient at 12½ years, immediately before oral diethyl-stilbestrol therapy. D. Patient 6 months after diethyl-stilbestrol, in daily doses of 5 mgm., was started. Note breast and nipple development.

size. The labia increased in size. There was no definite change in the hirsutism but the acne retrogressed. Patient J. W. (Case 10) did not have any nausea or menstrual bleeding during the period when diethyl-stilbestrol therapy alone was given. Furthermore, discontinuation of this therapy did not result in menstruation. However, 3 days after a 10-day period during which she was given 60 mgm. of anhydro-hydroxy-progesterone¹⁰ in addition to 5 mgm. of diethyl-stilbestrol by mouth per day, she had a 3-day period of menstrual flow. On the other hand, patient C. O.

(Case 6), after receiving only diethyl-stilbestrol for a period of 3 months, had 2 periods of menstrual bleeding, lasting 4 days each. The personality of these girls also underwent an apparent change. Before therapy was started, these children were morose and reticent and gave the impression that they were self-conscious and unhappy. At about the time that breast development first became evident, they became more effective individuals as evidenced by a more cheerful disposition, a more self-confident demeanor, and, particularly, an unexpected improvement in school work.

During a control period of 3 or more days,

¹⁰ We are indebted to Dr. M. Gilbert of the Schering Corporation for a generous supply of this material.

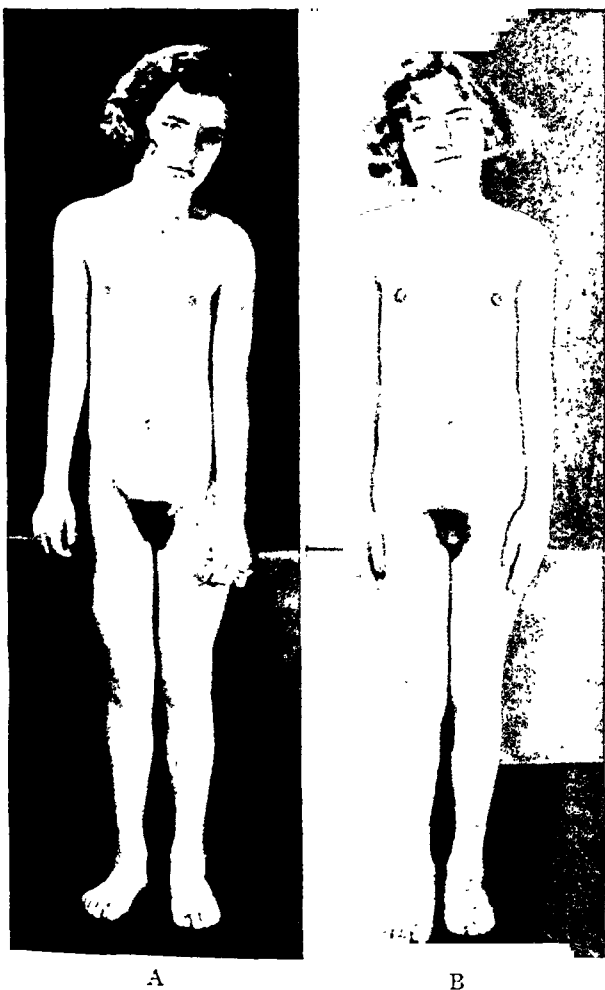


FIG. 9. CASE 6 (C. O.). ADRENAL CORTICAL VIRILISM PROBABLY DUE TO HYPERPLASIA OF THE ADRENAL CORTEX

A. Patient at 9 years, immediately before therapy. B. Patient after 5 to 10 mgm. of diethylstilbestrol had been given daily by mouth for 6 weeks. Note enlargement of breasts and nipples with pigmentation of areolae of nipples.

preceding the administration of diethylstilbestrol and at various intervals after the drug was started, urine samples representing 24-hour intervals of time were collected and assayed for their total 17-ketosteroid content. The results obtained on the foregoing 2 girls and on a boy (R. P., Case 2) with adrenal cortical hyperplasia are given in Figure 10. The abscissa shows the time intervals. The height of the solid black areas along the abscissa depicts the milligrams per day of diethylstilbestrol, as given by the stilbestrol ordinate scale. The height of the circles and crosses, con-

nected by the lines, gives the total 17-ketosteroid output per day, corresponding to the 17-ketosteroid ordinate scale. The double vertical line running through the initial (average control) 17-ketosteroid value given for each patient indicates the range of values obtained during the control period.

The figure shows that there is a tendency for the total 17-ketosteroid output to diminish during periods when diethylstilbestrol is being given, and for the level to rise when the therapy is discontinued. However, the changes in 17-ketosteroid levels are neither striking nor consistent.

DISCUSSION

The data presented indicate that quantitative analysis of specific fractions of the urinary 17-ketosteroids reveals more striking differences between patients with adrenal cortical hyperplasia and adrenal cortical carcinoma than does assay of the total 17-ketosteroids alone. Whereas the pattern of the 17-ketosteroids excreted by individuals with hyperplasia of the gland corresponds roughly to the normal, the pattern observed for individuals with carcinoma of the adrenal cortex veers widely from the normal. This abnormal pattern appears to be characteristic of carcinoma of the adrenal cortex, whether associated with adrenal cortical virilism or Cushing's syndrome. Thus there appears to be an alteration in biochemical function, corresponding to the differences in the cellular morphology of hyperplastic and of carcinomatous adrenal cortices. The importance of these observations in differential diagnosis is clear.

The observations on the clinical progress of children with adrenal cortical hyperplasia raise questions of interest from the point of view of therapy. The enlargement of the testes of the boy patient (Case 9) suggests that the precocious masculinization of hyperplasia may not be a serious complaint for boys. If this gonadal growth and gradual transition from precocious virilism to seemingly normal maturation is characteristic of male patients with the disease, it may explain the low recorded incidence of adrenal cortical virilism in adult males. However, it is possible that the adrenal cortical androgens upset the normal pituitary-gonadal relationships, thereby causing a disturbance in gonadal function which has not as yet been recognized.

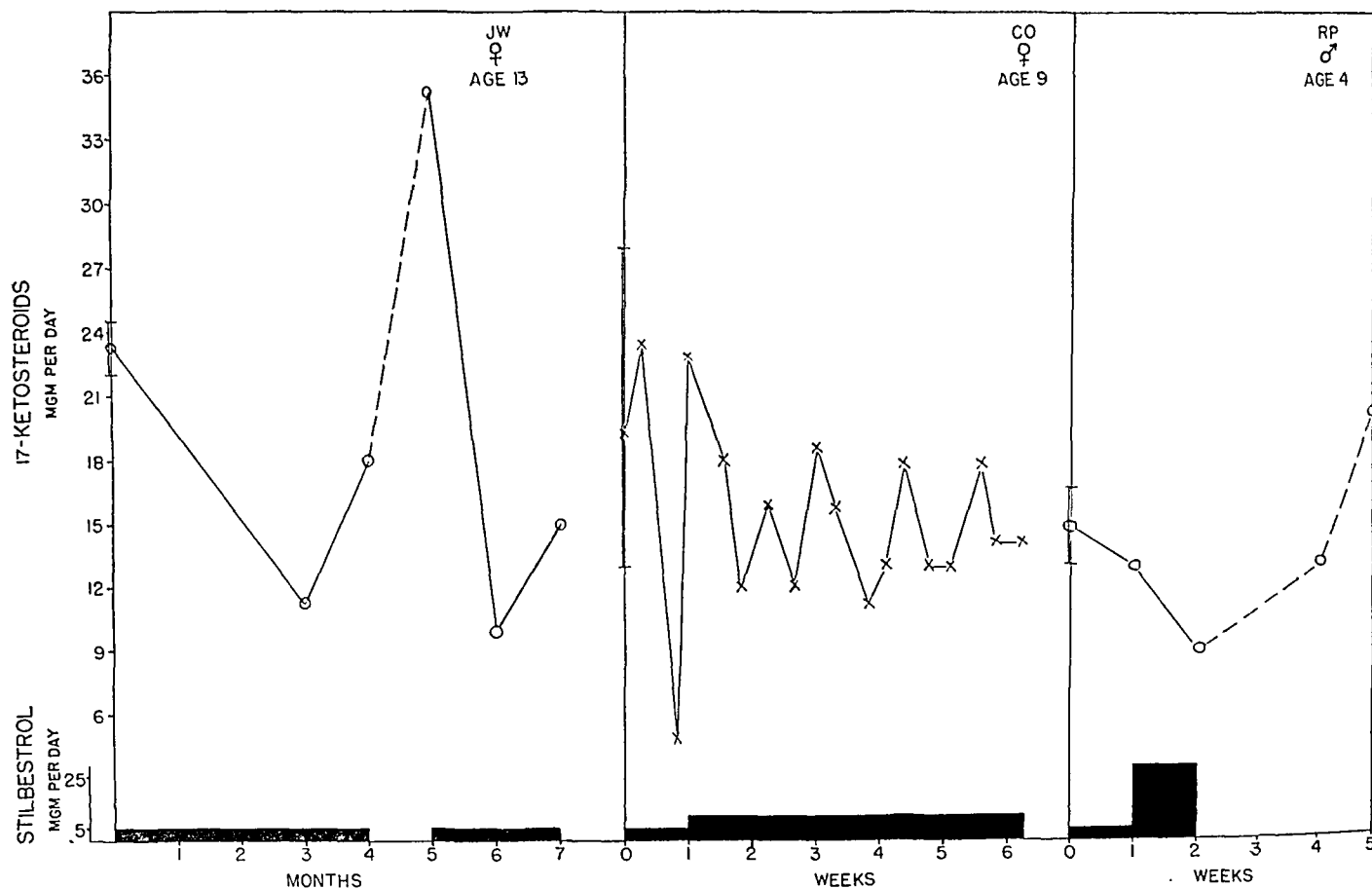


FIG. 10. INFLUENCE OF ORALLY ADMINISTERED DIETHYL-STILBESTROL ON TOTAL URINARY 17-KETOSTEROID EXCRETION PER DAY BY 3 CHILDREN WITH HYPERPLASIA OF THE ADRENAL CORTEX

J. W. is case 10; C. O. is case 6b; and R. P. is case 2. The duration of therapy is given by the abscissa. The dosage of diethyl-stilbestrol is shown by the height of the solid sections at the bottom of the figure. The total 17-ketosteroid output is given by the circles and crosses. The double vertical line at the left hand of each section indicates the range in 17-ketosteroid values during a control period. A solid line connecting the circles shows that diethyl-stilbestrol was being given; a broken line represents periods when it was not being given.

On the other hand, the prognosis for girls with virilism due to adrenal cortical hyperplasia is much less favorable. The tendency to masculinization persists and no normal feminine maturation takes place during the adolescent age period or later. Because the hyperplastic adrenal cortical tissue is believed to be the source of the excess androgenic hormones responsible for the masculinization in these patients, attempts to alleviate the condition by partial adrenalectomy were logical. Unfortunately, however, subtotal adrenalectomy has not proved to be permanently effective. This finding suggests that the adrenal cortical hyperplasia and hyperactivity may not reflect a primary disease of the adrenal cortex, but may result from adrenal cortical stimulation by nervous or humoral agents such as pituitary adrenocorticotrophic hormones. Complete adrenalectomy is, of course, incompatible

with life. This failure of surgical therapy led to the therapeutic trial of diethyl-stilbestrol, which is a potent, inexpensive, and apparently non-toxic estrogen. It would appear that the drug is capable of transforming girls with the masculinization of adrenal cortical virilism into persons with predominantly feminine characteristics. This is probably a purely cosmetic transformation, unaccompanied by the changes in ovarian function necessary for fertility or by the striking changes in adrenal cortical activity which would have been suggested by definite alterations in the urinary 17-ketosteroid output. It would thus appear that the feminization resulted from the artificial increase in the concentration of circulating estrogenic hormones. A continuation of this feminine development would, therefore, seem to depend on prolonged estrogen therapy.

There is experimental evidence that estrogens may cause hypertrophy of the adrenal cortex (20) and that estrogen therapy may not be physiologically ideal. For this reason the effectiveness of agents which may produce atrophy of the adrenal cortex (20, 21) should be investigated as such agents become available for clinical use.

SUMMARY AND CONCLUSIONS

The total urinary 17-ketosteroid excretion is abnormally increased in patients with adrenal cortical hyperplasia or adrenal cortical carcinoma.¹¹ However, the magnitude of the total 17-ketosteroid excretion is not always a reliable index of the exact nature of this hyperactivity. On the other hand, the urinary output of the beta-alcoholic and the non-alcoholic 17-ketosteroids has been found to be markedly elevated in patients with adrenal cortical carcinoma, whereas their output was either normal or but slightly elevated in patients with cortical hyperplasia. Assay of the excretion of these latter 2 fractions therefore appears to be of diagnostic value in differentiating patients with adrenal cortical hyperplasia from those with carcinoma of the adrenal cortex.

In agreement with the observations of others (22 to 24) the urinary pregnanediol values are not consistently elevated in patients with adrenal cortical hyperplasia.

Adrenal cortical virilism, when due to hyperplasia of the adrenal cortex, usually persists without a tendency to spontaneous remissions. Children of both sexes may tend to grow rapidly in the early stages of the disease, but cease growing at an abnormally early age because of premature closure of the epiphyses. There is a probability that the boys will become normal appearing adult males but whether or not they will attain fertility is uncertain. If left untreated, the girls tend to remain chronically masculinized.

¹¹ Unpublished observations are available on eight children with sexual precocity apparently due either to accelerated normal physiologic processes or to central nervous system lesions. The sexual precocity in these children differed from that observed in the adrenogenital syndrome in that the gonads rather than the adrenal cortex appeared to be the source of the sex hormones responsible for the secondary sex development. The total 17-ketosteroid values of this group were not markedly elevated, but corresponded approximately to values obtained on normal individuals of the same physiologic age.

The treatment of choice for patients with a carcinoma of the adrenal cortex is surgical removal. On the other hand, surgery does not appear to be indicated in the treatment of patients with adrenal cortical hyperplasia. In girls with this latter disease, orally administered diethylstilbestrol causes development of the female secondary sexual characteristics and improvement in psychological outlook. On the other hand, there appears to be little indication for such therapy in the male.

Addenda: Since this paper went to press, additional measurements have been made on 2 adult women with adrenal cortical virilism. The first patient had proven adrenal cortical hyperplasia. She excreted a total of 46.8 mgm. of 17-ketosteroids per day. Of these, 6.4 mgm. were beta-alcoholic 17-ketosteroids. The second patient had a proven adrenal cortical carcinoma. Her total 17-ketosteroid output was 74.0 mgm. per day. Of these, 28.5 were beta-alcoholic 17-ketosteroids.

BIBLIOGRAPHY

1. Grollman, A., *The Adrenals*. Williams and Wilkins Co., Baltimore, 1936.
2. Haymaker, W., and Anderson, E., The syndrome arising from hyperfunction of the adrenal cortex: The adrenogenital and Cushing's syndromes—A review. *Internat. Clin.*, 1938, 4, 244.
3. Dorfman, R. I., Wilson, H. M., and Peters, J. P., Differential diagnosis of basophilism and allied conditions. *Endocrinology*, 1940, 27, 1.
4. Lukens, F. D. W., and Palmer, H. D., Adrenal cortical virilism. *Endocrinology*, 1940, 26, 941.
5. Crooke, A. C., and Callow R. K., The differential diagnosis of forms of basophilism (Cushing's syndrome) particularly by estimation of urinary androgens. *Quart. J. Med.*, 1939, 8, 233.
6. Callow, R. K., Isolation of the male hormone present in the urine of a patient with an adrenal tumor. *Chem. and Ind.*, 1936, 55, 1030.
7. Butler, G. C., and Marrian, G. F., Chemical studies on adrenogenital syndrome. I. The isolation of 3 (α)-hydroxyetioallocholane-17-one, 3 (β) hydroxyetioallocholane-17-one (isoandrosterone), and a new triol from the urine of a woman with an adrenal hyperplasia. *J. Biol. Chem.*, 1938, 124, 237.
8. Hirschmann, H., Isolation of isoandrosterone from urine in a case of virilism. *Proc. Soc. Exper. Biol. and Med.*, 1941, 46, 51.
9. Burrows, H., Cook, J. W., Roe, E. M. F., and Warren, F. L., Isolation of Δ^4 -androstadiene-17-one from the urine of a man with a malignant tumor of the adrenal cortex. *Biochem. J.*, 1937, 31, 950.

10. Wolfe, J. K., Fieser, L. F., and Friedgood, H. B., Nature of androgens in female adrenal tumor urine. *J. Am. Chem. Soc.*, 1941, 63, 582.
11. Talbot, N. B., Berman, R. A., MacLachlan, E. A., and Wolfe, J. K., The colorimetric determination of neutral steroids (hormones) in a 24-hour sample of human urine (pregnanediol; total, alpha and beta alcoholic, and non-alcoholic 17-ketosteroids). *J. Clin. Endocrinol.*, 1941, 1, 668.
12. Talbot, N. B., Butler, A. M., and MacLachlan, E. A., Alpha and beta neutral ketosteroids (androgens). Preliminary observations on their normal urinary excretion and the clinical usefulness of their assay in differential diagnosis. *New England J. Med.*, 1940, 223, 369.
13. Broster, L. R., Gardiner Hill, H., and Greenfield, J. G., The adrenogenital syndrome associated with cortical hyperplasia; the results of unilateral adrenalectomy. *Brit. J. Surg.*, 1932, 19, 557.
14. Lissner, H., Feminization and demasculization of a 17-year-old girl by injections of stilbestrol. *Endocrinology*, 1940, 27, 385.
15. Fraser, R., Forbes, A. P., Albright, F., Sulkowitch, H. W., and Reifstein, E. C., Jr., Colorimetric assay of 17-ketosteroids in the urine. *J. Clin. Endocrinol.*, 1941, 1, 234.
16. Baumann, E. J., and Metzger, N., Colorimetric estimation and fractionation of urinary androgens. *Endocrin.*, 1940, 27, 664.
17. Talbot, N. B., Berman, R. A., and MacLachlan, E. A., Elimination of errors in the colorimetric assay of neutral urinary 17-ketosteroids by means of a color correction equation. *J. Biol. Chem.*, 1942, 143, 211.
18. Normal standards of height and weight for children from birth to six years were obtained from the Department of Child Hygiene, Harvard School of Public Health and for children from 6 to 16 years from studies of the University of Iowa.
19. Todd, T. W., *et al.* Atlas of skeletal maturation. C. V. Mosby Co., St. Louis, 1937.
20. Selye, H., Compensatory atrophy of the adrenals. *J. A. M. A.*, 1940, 115, 2246.
21. Wells, B. B., and Kendall, E. C., A qualitative difference in the effect of compounds separated from the adrenal cortex on distribution of electrolytes and on atrophy of the adrenal and thymus glands of rats. *Proc. Staff Meet., Mayo Clin.*, 1940, 15, 133.
22. Venning, E. H., Weil, P. G., and Browne, J. S. L., Excretion of sodium pregnanediol glucuronide in the adrenogenital syndrome. *J. Biol. Chem.*, 1939, 128, cvii.
23. Finkler, R. S., Pseudohermaphroditism, Pregnanediol glucuronide excretion. *J. Clin. Endocrinol.*, 1941, 1, 151.
24. Salmon, U. J., Geist, S. H., and Salmon, A. A., Excretion of pregnanediol in women with virilism. *Proc. Soc. Exper. Biol. and Med.*, 1941, 47, 279.

AN ELECTROPHORETIC STUDY OF THE PROTEIN COMPONENTS IN CEREBROSPINAL FLUID AND THEIR RELATION- SHIP TO THE SERUM PROTEINS¹

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Increases in the total protein content of cerebrospinal fluid frequently occur in diseases of the nervous system, and the determination of total protein content is one of the most useful diagnostic laboratory procedures to the neurologist (1). The origin of the cerebrospinal fluid protein is usually considered to be the blood plasma and the increases in total protein content in meningitis are believed due to transudation of serum through the walls of inflamed vessels (1). In other conditions, such as polyneuritis, the reason for the elevated protein content is not clear (1). There are few data available regarding the composition of the cerebrospinal fluid proteins, chiefly because the small amounts of protein present make chemical studies difficult. Freund (2) has shown that the titre of antibody in the spinal fluid of passively immunized rabbits is about 1/300 that of the serum, which is of the same order as the ratio of the total protein of spinal fluid to that of serum. By the use of the Tiselius electrophoresis technic (3), the authors were able to demonstrate that the electrophoretic patterns of concentrated cerebrospinal fluids were similar to those of serum (4). The present report covers a more extensive study of the effect of variations in the serum proteins on the composition of the cerebrospinal fluid proteins, and of the changes in the proportions of the cerebrospinal fluid proteins in various neurological conditions. The fraction of cerebrospinal fluid associated with abnormal colloidal gold activity is identified, and the inhibiting effects of the albumin fraction on the colloidal gold reaction are considered.

EXPERIMENTAL

Cerebrospinal fluids were obtained from patients on whom encephalography or ventriculography was per-

formed. Volumes of cerebrospinal fluid as large as 80 ml. were obtained in this manner. Samples were taken for the colloidal gold test and for determination of the total protein content. The rest of the fluid was then placed in a cellophane dialyzing membrane with a one hole rubber stopper at one end and a solid rubber stopper at the other. The membrane was connected by rubber and glass tubing to a small tank of nitrogen, and a gas pressure of 5 pounds per sq. inch (about 250 mm. Hg) was constantly maintained on the inside of the membrane. The membrane was immersed in saline so that it was completely covered and the entire apparatus was kept in the icebox at about 5° C. Under these conditions it was found possible to concentrate the cerebrospinal fluids from 70 ml. to about 2 ml. in about 2 to 3 days. Several fluids may be concentrated simultaneously by the use of a "Y" tube connecting to the nitrogen tank. It is necessary to test the membranes by applying 5 pounds pressure for several hours before adding the spinal fluid to avoid loss of the specimen in weak membranes or through the connections. After the fluid is concentrated to the desired volume, it is redialyzed against a solution containing 0.15 M NaCl + 0.02 M phosphate buffer at pH 7.4 and is studied in the Tiselius electrophoresis apparatus, using a microcell of 2 ml. capacity.

Serum samples were diluted about 1:4 and dialyzed against the same saline phosphate buffer used for the spinal fluids.

The Tiselius electrophoresis cell (3) consists of a U-tube having a rectangular cross-section, the end walls of which have high optical quality so that light refraction caused by concentration gradients in the solution can be detected accurately. The concentrated cerebrospinal fluid is placed in the bottom half of the U-tube underneath a buffer against which it has been previously dialyzed until its conductivity and pH have assumed approximately the same value as that of the buffer. To facilitate filling and recovery of material after separation, the electrophoresis cell (U-tube) is divided into sections with sliding flange plates, so that each section may be sealed off from the rest of the system. The tops of the U-tube connect to large buffer vessels containing electrodes. The whole is placed in a water bath thermostatically maintained at a low temperature (1.5° C. in our work). A boundary is formed by aligning the section containing the protein solution with the rest of the cell. A regulated voltage applied to the electrodes causes a constant current to flow. Convectional disturbances due to heat are mini-

¹ Aided in part by a grant from the William J. Matheson Commission.

² Dr. Landow died on March 27, 1942.

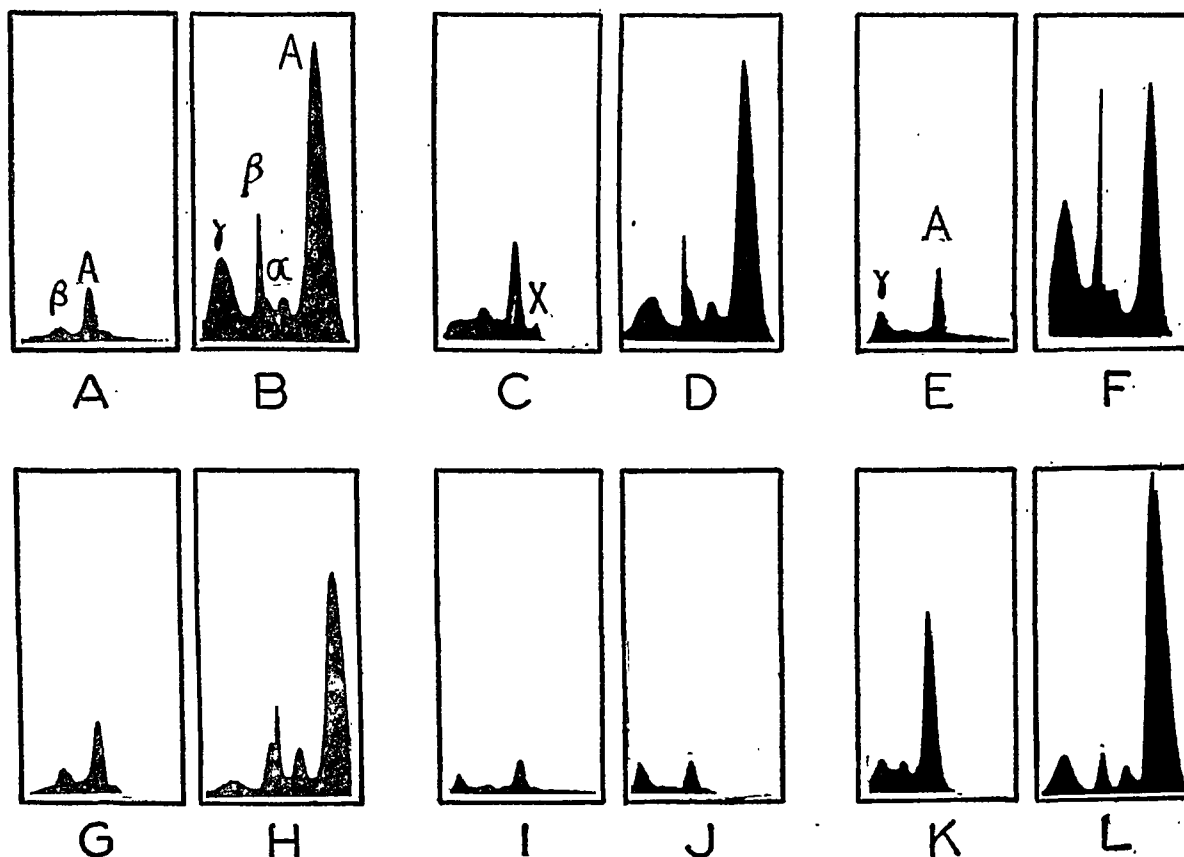


FIG. 1. ELECTROPHORETIC PATTERNS

Case number 661981—Idiopathic grand mal (normal)	A Spinal fluid	B Serum
647635—Anxiety state (normal)	C Spinal fluid	D Serum
652282—Lymphopathia venereum	E Spinal fluid	F Serum
661095—Multiple myeloma	G Spinal fluid	H Serum
WH 138—Multiple sclerosis	I Spinal fluid	
655751—Neurosyphilis	J Spinal fluid	
635202—Diabetic neuritis	K Spinal fluid	
652371—Left frontal cystic astrocytoma	L Cyst fluid	

mized by maintaining the mean temperature (temperature is highest at center and lowest at walls of cells) of the solution at the point where the change of density with temperature is least (temperature of maximum density). Convection limits the wattage which may be dissipated in the cell.

The current produces an electrical field throughout the cell. If the cell has a uniform cross-sectional area, $A \text{ cm}^2$, the field E equals I/KA where I is the current in amperes and K is the specific conductivity of the solution in reciprocal ohms. This electrical field causes the different protein components of the solution to migrate at a rate proportional to the statistical surface charge per unit area of the particles or molecules, and in a direction determined by the sign of this charge. This rate of migration, in cm. per second per unit field in volts per cm., is designated as the mobility of the component.

Bending of light rays caused by concentration gradients at the boundaries of each component may be detected by various optical methods; in this study, the Toepler "Schlieren" method, as modified by Longsworth (5), was used. With this method each electrophoretically distinct

protein component gives a symmetrical curve, the area of which is the total refractive index change and is proportional to the concentration of the protein component. By integrating the area for each component in the patterns (Figure 1), the relative concentrations may be obtained, assuming that the specific index of refraction of all components is the same.

After the migration is complete, samples of pure individual components may be removed from the U-tube and tested for colloidal gold activity, etc.

The total protein content of the cerebrospinal fluid was determined turbidimetrically by precipitation with sulfosalicylic acid. The concentration of any electrophoretic component in the original spinal fluid was obtained by multiplying the total protein content of the fluid by the percentage composition of that component as calculated from the electrophoretic pattern.

The colloidal gold tests were carried out in the usual manner, by addition of 2.5 ml. colloidal gold solution to 0.5 ml. of spinal fluid dilutions of 1:10, 1:20, 1:40 . . . 1:5120. The tubes were allowed to stand at room temperature and read after 24 hours. The color changes

TABLE I
Mobilities and concentrations of protein components in cerebrospinal fluid and in serum

Diagnosis		Vol. of fluid used	pH	Mobility $\mu \times 10^3$					Per cent composition							Concentration in original spinal fluid					Colloidal gold reaction on original spinal fluid
				α	β	ϕ	γ	χ	α	β	ϕ	γ	A/G	Total protein (found)	mgm. per 100 ml.						
															A	α	β	γ			
																			A	α	
661981	Idiopathic grand mal	65	7.4	5.5	2.8		0.9(5)		71.1	22.6		6.3	2.5	27	19	6	2	1100000000			
661981 (serum)		7.4	5.2	3.7	2.6		0.8		60.7	6.5	12.6	20.2	1.5	23	15	5	2	1100000000			
371706	Anxiety state	63	7.4	5.5	4.1	2.9	0.9	5.2	67.3	5.1	20.4	7.2	2.1	23	15	5	2	1100000000			
661917	Idiopathic cortical seizures	67	7.4	4.9	3.4	2.7	0.7		61.0	8.5	13.5	14.1	1.6								
661917 (serum)		7.4	6.2	3.4	1.5	0.9		62.4	26.2	5.4	11.3	1.7	30	19	7	2	3	1100000000			
647635	Anxiety state	60	7.4	4.8	3.2	2.2	0.7		67.3	9.9	8.8	14.0	2.1								
647635 (serum)			7.4	8.1	6.0	3.2	1.1	7.6	58.4	25.8		8.2	1.7	38	22	10	3	1100000000			
652282	Lymphopathia venereum	16	7.4	5.1	3.8	2.7	0.9		61.0	7.9	6.1	17.3	1.6								
652282 (serum)			7.5	5.9	3.1			56.3	12.0		31.8	1.3	90	51	11	29	1111222100				
643966	Lymphopathia venereum	25	7.3	5.0	3.2	2.3	0.6		39.6	8.4	17.7	34.3	.7								
655646				5.5	4.1	2.7	0.4		42.4	9.4	21.9	26.5	0.7								
655646 (serum)	Neurosphilis	34	7.3	4.8	3.3	2.9	1.6* 0.5		34.0	3.6	3.3	11.7	38.4	0.5							
660660				5.4	4.7	2.5			49.6	5.0	7.2	38.2	1.0	55	27	3	4	1100000000			
660660 (serum)	Cirrhosis	25	7.3	5.4	3.6	2.9	0.8		60.7	12.3	5.2	11.0	1.6								
657549				5.7	2.8				50.6	19.5		29.9	1.0	37	19	7	11	1100000000			
657549 (serum)	Lucas; arsenical encephalitis	11.5	7.4	5.5	3.7	2.6	1.2* 0.8		36.0	6.9	11.4	9.3	10.8	.6							
661095				5.3	2.5		0.5		61.4	13.7		25.0	1.6	92	56	13	23	1112211000			
661095 (serum)	Multiple myeloma	33	7.4	4.9	3.1	2.6	0.5		45.2	9.1	13.8	31.9	.8								
				5.4	2.5				66.4			33.7		38	25	13		0000000000			
				5.4	3.7	2.5			67.9	9.9	7.5	4.9									

* Not fibrinogen.
Where two values are given, the values refer to mobility or composition of α_1 and α_2 or β_1 and β_2 components.

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TABLE III
Colloidal gold curves on electrophoretic fractions of concentrated spinal fluids

Case number	Diagnosis	Colloidal gold curves			
		Original un-concentrated spinal fluid	Albumin	Middle	Gamma
661981	Idiopathic grand mal	1100000000	0000000000	0000000000	1112210000
661917	Idiopathic cortical seizures	1100000000	0000000000	0000000000	3321000000
647635	Anxiety state	1100000000	0000000000		0000000000*
658958	Post traumatic headache	1100000000	0000000000	0000000000	0000000000
635202	Polyneuritis	1100000000	0000000000	0000000000	1112332210
660660	Cirrhosis	1110000000	0000000000	1111222110	1243210000
661808	Multiple sclerosis	1100000000	0000000000	2112221100	5432100000
WH 138	Multiple sclerosis	2222100000	0000000000	3322221100	5553321000
655751	Neurosyphilis	5555544210	0000000000	5554332110	5555432100
659043	Neurosyphilis	1122100000	0000000000	3332233321	5554431100
657549	Lues; arsenical encephalitis	1112211000	0000000000	1111222210	5555432100
659002	Neurosyphilis	1111122211	0000000000	1112221000	5554321000

* Whole globulin.

the albumin had an inhibiting effect on the colloidal gold reaction. In gamma globulin samples obtained from sera showing colloidal gold changes, it was possible completely to abolish the colloidal gold reaction by adding albumin. Details will be published in a separate communication.

DISCUSSION

The observation that changes in the electrophoretic patterns of the spinal fluid are influenced by changes in the composition of the serum proteins strongly indicates that much of the spinal fluid protein is derived from the blood. The order of magnitudes of the ratios of spinal fluid gamma globulin to serum gamma globulin are in agreement with the average of 1:300, found by Freund, for the distribution of antityphoid agglutinins between the spinal fluid and serum of rabbits. This is not surprising as human antipneumococcus antibody and rabbit antibodies to several types of antigens were found to have the same molecular weights as their respective gamma globulins (6), and hence would be distributed on both sides of the hemato-encephalic barrier to the same extent.

The data obtained in neurosyphilis (case 655646), however, suggest that not all of the spinal fluid protein is derived from the blood. In this case, the A/G ratio of the spinal fluid was much lower than that of the blood and the percentage of gamma globulin was much higher than that in the serum. This spinal fluid also showed

colloidal gold changes which are associated with increases in the gamma globulin. The data would suggest that some formation of gamma globulin could take place within the tissues of the central nervous system and be poured into cerebrospinal fluid, since it is difficult to imagine an altered permeability of the hemato-encephalic barrier which could produce an increase in gamma globulin without producing the same or an even greater increase in the smaller albumin molecule. The formation of some gamma globulin in the central nervous system is in accord with the views of Katzenelbogen (7) that cerebrospinal tissues are capable of producing antibodies, and that the origin of the antibodies in the cerebrospinal fluid is twofold, from the blood and from the cerebrospinal tissues. It also agrees with the views of Sabin (8) who has suggested that antibodies and normal gamma globulin are formed in the cells of the reticulo-endothelial system by a partial shedding of their surface cytoplasm. This hypothesis will explain the electrophoretic data as well as the fact that positive Wasserman reactions may be obtained frequently in spinal fluid, but not in the serum, in cases of neurosyphilis.

SUMMARY

1. The electrophoretic pattern of cerebrospinal fluid resembles that of serum.
2. Alterations in the composition of the protein components of serum are reflected in the cerebrospinal fluid, but the changes are not as marked.

In neurosyphilis, however, an increased gamma globulin occurs in cerebrospinal fluid, without similar changes in the blood stream.

3. Colloidal gold activity is associated with the gamma globulin fraction, and albumin has an inhibiting effect on the colloidal gold reaction.

The authors are indebted to Drs. Tracy J. Putnam and Robert F. Loeb for many suggestions and for assistance in obtaining material. The colloidal gold tests were carried out by Miss Ruth Shivitz of the Department of Bacteriology, and Misses Sheila Goldsmith and Helen Sikorski assisted in the electrophoretic experiments.

BIBLIOGRAPHY

1. Merritt, H. H., and Fremont-Smith, F., *Cerebrospinal Fluid*. W. B. Saunders Co., Philadelphia, 1937.

2. Freund, J., Accumulation of antibodies in the central nervous system. *J. Exper. Med.*, 1930, 51, 889.
3. Tiselius, A., A new apparatus for electrophoretic analysis of colloidal mixtures. *Tr. Faraday Soc.*, 1937, 33, 524.
4. Kabat, E. A., Landow, H., and Moore, D. H., Electrophoretic patterns of concentrated cerebrospinal fluid. *Proc. Soc. Exper. Biol. and Med.*, 1942, 49, 260.
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BIBLIOGRAPHY

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OBSERVATIONS ON THE NATURE OF MYASTHENIA GRAVIS. THE EFFECT OF THYMECTOMY ON NEURO- MUSCULAR TRANSMISSION¹

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This communication reports a study of the state of neuromuscular transmission, and the effects upon it of prostigmine and acetylcholine injected into the brachial artery, in five patients with severe myasthenia gravis, five months after the total extirpation of the thymus gland. The partial block in neuromuscular transmission and the abnormal reactions to the intra-arterial injection of prostigmine which existed pre-operatively (1, 2) have been altered profoundly. The results of these experiments indicate that in myasthenia gravis the thymus influences greatly the function of the motor nerves and the striated muscles which they innervate. These findings furnish objective evidence of an action of the thymus gland, and an analysis of the results may assist in understanding the normal physiological function of this structure.

In addition to changes in the physiological and pharmacological patterns of neuromuscular function which followed thymectomy, there occurred in three of the patients an extraordinary clinical improvement which developed concomitantly with the reversion of neuromuscular function toward normal.

METHODS

The methods employed in this study have been described in detail previously (3). Injections of prostigmine and acetylcholine were made into the brachial artery. The ulnar nerve was stimulated through the intact skin above the elbow, by square waves lasting about one millisecond and presented singly, in pairs, or in salvos by the delay circuits of Marshall and Talbot (4). These stimuli, transformer-coupled to the stimulating electrodes, had an amplitude greater than necessary to elicit maximum action potentials from the muscle electrodes placed on the skin over the *m. abductor digiti quinti*. These muscle action potentials were led to the input of a condenser-coupled amplifier and recorded by means of a cathode ray oscillograph.

¹ Aided in part by a grant from the John and Mary R. Markle Foundation.

MATERIAL

The clinical histories of these patients have been recorded before (5).

Patient P. C. (Unit #225775). A thirty-three-year-old colored farmer developed severe and progressive myasthenia gravis in July 1940. He required 240 mgm. of prostigmine a day before operation and even then was incapacitated. The thymus was removed July 26, 1941. By August 9, 1941, he no longer required prostigmine. Since then there has been a steady return of strength and by February 1942, he was able to perform light work.

Patient R. L. (Unit #255345). A twenty-eight-year-old graduate student developed myasthenia gravis in August, 1940. By July 1941, she was confined to bed because of weakness and required 240 to 270 mgm. of prostigmine a day. The thymus was removed on August 4, 1941. She required no further prostigmine after August 18, 1941. By February 1942, she was working eight hours a day in the statistical department and the only residual evidence of former myasthenia was weakness of the muscles of the pelvic girdle, which made it necessary for her to walk with a wide base.

Patient L. K. (Unit #127185). A twenty-two-year-old colored woman who developed severe myasthenia gravis in 1935. In August 1941, her activities were greatly limited by weakness, despite the oral administration of 120 mgm. of prostigmine a day. The thymus was removed August 18, 1941. When discharged from the hospital in October 1941, she was walking and eating easily without any medication. In February 1942, she showed evidences of continued gain in strength. She felt that she required 7.5 mgm. of prostigmine in the morning on arising, and occasionally if she went out in the evening, she would take 15 mgm. of prostigmine. Her activity was not limited by weakness.

RESULTS

The response to a single maximal nerve stimulus

The characteristic electromyogram of the *m. abductor digiti quinti*, elicited by a maximal stimulus to the ulnar nerve, in normal subjects is marked by its uniformity. In any one individual, the voltage of the response never varies more than 15 per cent from day to day; in most instances, the variation is less than 5 per cent (6). This

constancy has been interpreted to indicate that under the conditions of the examination in the normal subject, all excitable muscle fibers respond to a single maximal motor nerve stimulus.

In contrast to the normal subject, the patient with severe myasthenia gravis exhibits an electromyogram which indicates that some of the available muscle fibers do not respond to a maximal nerve stimulus. This is suggested by the small amplitude of the action potential and by the effect of prostigmine which improves the muscle strength and increases the voltage of the responses. The reduction in amplitude of the action potential in the untreated myasthenic state is proportional to the number of muscle fibers which fail to respond to a maximal nerve stimulus (1).

In two of these patients we have had the oppor-

tunity of studying the state of neuromuscular transmission before and after thymectomy. In

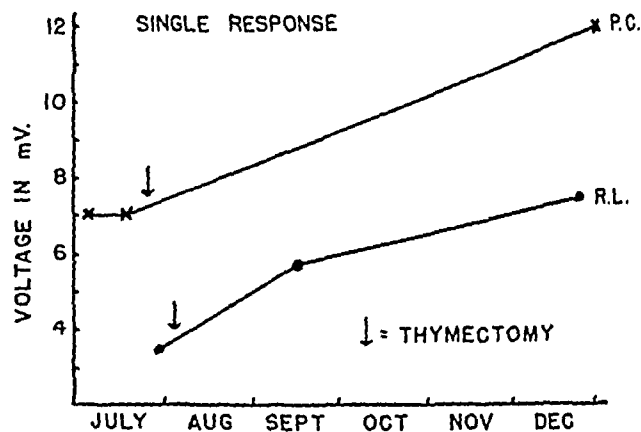


FIG. 1. THE VOLTAGE OF THE RESPONSE OF THE *M. Abductor Digiti Quinti* TO A SINGLE MAXIMAL STIMULUS TO THE ULNAR NERVE INCREASES AFTER THYMECTOMY Patients R. L. and P. C.

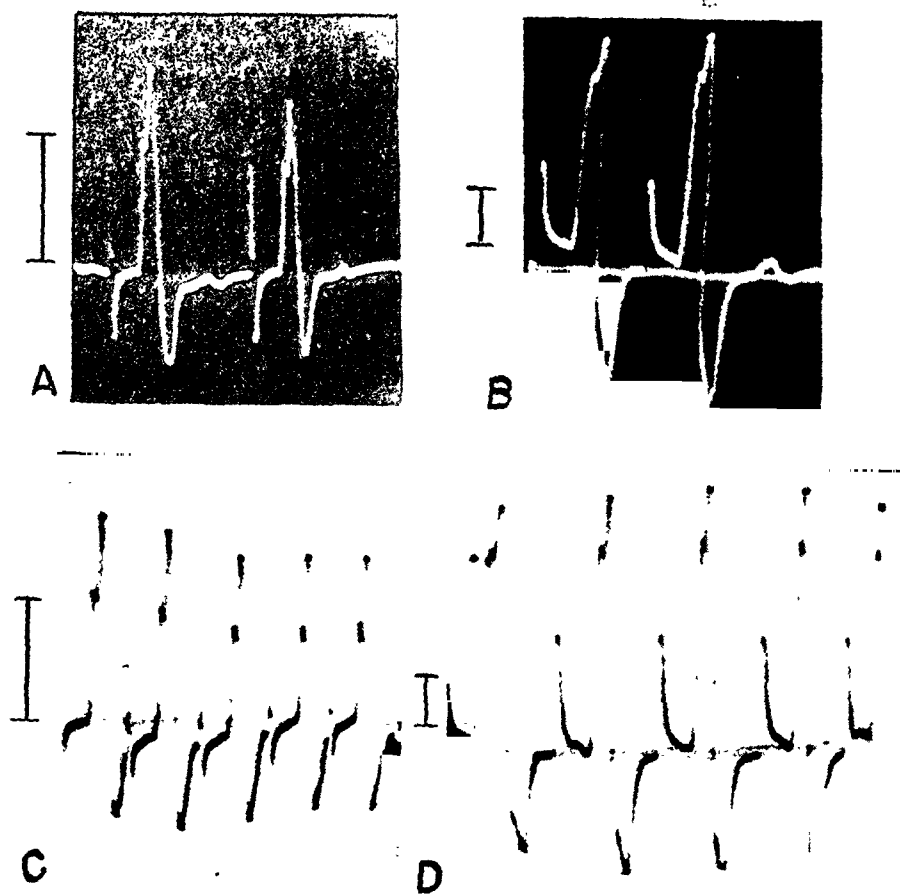


FIG. 2. PATIENT R. L.

A. Before thymectomy, the depression of the second response which follows at an interval of 32 msec. is 20 per cent. B. Five months after thymectomy, the second response at a 20 msec. interval shows slight facilitation. C. Before thymectomy, there is progressive depression of the responses to a train of maximal stimuli at 16 msec. intervals; the fifth response is but 74 per cent of the first. D. Five months after thymectomy, a train of maximal stimuli at 20 msec. intervals results in facilitation. Calibration = 2.0 mV.

both instances, without any adjuvant therapy, there has occurred a large increase in the voltage of the response to a single maximal stimulus. In R. L. the voltage rose from 3.5 to 7.4 mV., an increase of 110 per cent; in P. C. the voltage rose from 6.9 to 12 mV., an increase of 70 per cent (Figure 1). These rises in potential, indicating an improvement in neuromuscular transmission, were accurate reflections of the clinical improvement.

*Transmission of paired maximal stimuli;
"the two-volley curve"*

In the normal subject, when two maximal stimuli, separated by a brief interval, are delivered to the motor nerve, the amplitudes of the corresponding muscle action potentials are equal (6). In these patients with severe myasthenia gravis, a depression of neuromuscular transmission, induced by the passage of the first volley across the junction, had resulted in a decline in the voltage of the second response of the pair (Figure 2A) (1, 7). Following thymectomy, the two-volley

response has been altered significantly. In the patient P. C., before operation, the voltage of the second response to a pair of stimuli delivered at an interval of 64 msec. had been only 40 per cent of the first. Five months after thymectomy, the voltage of the second response at the same two-volley interval was 82 per cent of the first (Figure 3). Thus the characteristic depression of neuromuscular conduction found during the myasthenic state had been greatly reduced.

In the patient R. L., the alterations in the two-volley curve which were noted five months after thymectomy were even more striking. Prior to operation, the depression of the second response to a pair of maximal stimuli, separated by a 33 msec. interval, had been 20 per cent. In contrast, after thymectomy the second response was actually greater in voltage than the first (Figure 2). This phenomenon of facilitation, which appeared in this patient during the postoperative period of improvement, may be compared with a similar process which we have recorded in another patient (1) during a spontaneous remission when myasthenia was minimal.

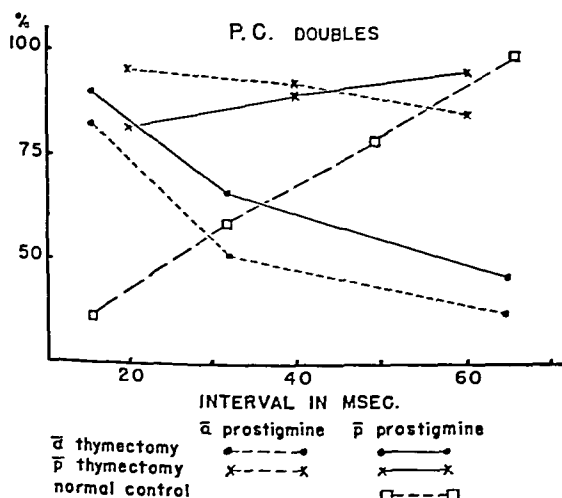


FIG. 3. TRANSMISSION CURVES OF PATIENT P. C.

The potential of the second response, expressed as percentage of the first, is plotted against the interval of the two stimuli, expressed in msec. *Before thymectomy:* ● ——— ● = marked depression and ● ——— ● the slight improvement after prostigmine. *After thymectomy:* x --- x indicates the depression of the second response which is minimal, and x — x shows that after prostigmine there is a different slope to the recovery curve, approaching that seen in the normal subject: □ --- □.

Transmission of a train of maximal stimuli

A salvo of maximal stimuli delivered to the ulnar nerve in a normal subject at 16 to 20 msec. intervals evokes a train of responses which are equal in voltage. In every myasthenic patient whom we have examined, this train of responses has been characterized by a progressive fall in the amplitude of the action potentials. The depression of the consecutive potentials has been roughly proportional to the severity of the myasthenia in the muscle examined; and the extent to which the defect in neuromuscular transmission has been repaired by prostigmine has also reflected the degree of clinical weakness.

Following thymectomy, the responses to the train of stimuli underwent a significant change in the direction of the normal response. In the patient P. C. before thymectomy, the fourth response to a train was depressed almost to extinction; and even after the intra-arterial injection of a large amount of prostigmine (1.5 mgm.), the fourth response was still only 41 per cent of the first. Five months after thymectomy, there had occurred a marked improvement in neuromuscular conduc-

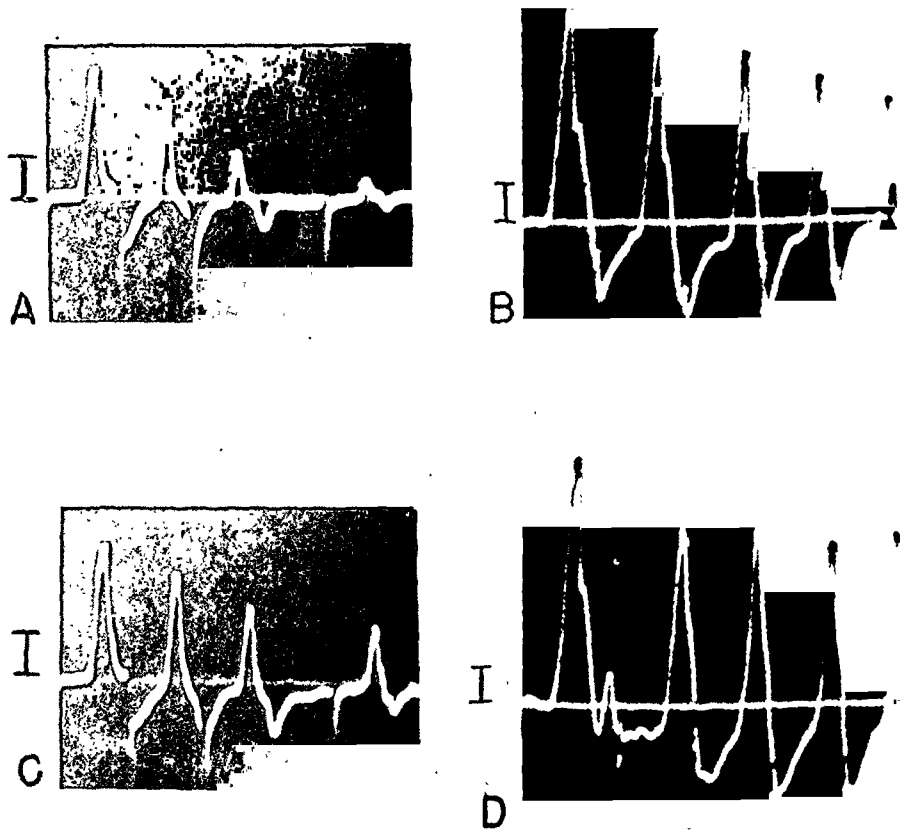


FIG. 4. PATIENT P. C.

A. Train of maximal stimuli at 16 msec. intervals before thymectomy. B. Same test five months after thymectomy at 20 msec. intervals showing rise in action potential and increased efficiency of neuromuscular transmission. C. Before thymectomy, showing the moderate improvement in transmission after injection of 2.0 mgm. prostigmine into the brachial artery. D. Same as B. but after 1.0 mgm. of prostigmine intra-arterially. Calibration = 2.0 mV.

tion, even though no prostigmine had been administered (Figure 4). The patient R. L. showed before thymectomy a characteristic progressive decrease in the voltages of the consecutive responses to a train of maximal stimuli. Five months after operation there was a complete reversal of the depressed response and a significant facilitation of the successive responses appeared (Figure 2). The occurrence of a similar phenomenon in another patient (B. W.) has been described before (1). The presence or development of facilitation may be a valuable criterion of the state of the myasthenic process, for in these two instances it has appeared during the development of a remission, the one spontaneous and the other apparently induced by extirpation of the thymus. A process of facilitation of this degree has never been seen in normal subjects, in whom the variations do not exceed 5 per cent.

The action of prostigmine

The effect on neuromuscular function of prostigmine injected into the brachial artery of normal subjects may be summarized briefly:

- (a) Profound paresis of the muscles in the injected extremity.
- (b) Fascicular twitching of the muscles in the injected extremity.
- (c) Repetitive action potential in response to a single nerve stimulus.
- (d) Depression of neuromuscular transmission, produced by the passage of one volley across the junction. This depression is greatest immediately after the first volley and disappears, in most instances, within 100 msec. (3) and therefore is of a different nature from the depression after activity in patients with myasthenia gravis.

The effect of prostigmine in the myasthenic patient is quite different and produces:

- (a) Partial or complete return of motor power.
- (b) No fasciculations.
- (c) No repetitive response despite enormous doses (2 to 3 nigr.).
- (d) Repair of the existing defect in neuromuscular transmission to or toward a normal response (1, 2).

These four differences in the effect of prostigmine on normal and myasthenic subjects were employed to estimate objectively the change in neuromuscular function which had occurred in the myasthenic patients following removal of the thymus.

Motor power. Before operation, five of the patients showed an increase in strength, locally and then generally, after the intra-arterial injection of prostigmine. The degree of improvement was roughly proportional to the initial weakness. In two patients, R. L. and L. K., who had regained almost normal strength after thymectomy, the intra-arterial injection of prostigmine in moderate doses (0.75 and 1.0 mgm.) produced weakness. In a third patient, P. C., who had had the severest myasthenia of the entire group and had experienced enormous clinical improvement after thymectomy, the injection of prostigmine into the brachial artery five months after the operation produced an increase in strength significantly greater than the same amount of drug (1.0 mgm.) had effected before operation. The remaining two patients, R. S. and M. W., whose myasthenia had been least severe and who had made virtually no clinical recovery, showed little or no change in this reaction to prostigmine, when tested two months after operation.

Fasciculation. None of these five patients had developed local or general fasciculations in response to the intra-arterial injection of prostigmine before thymectomy. All of these patients developed local fasciculations postoperatively. The extent and frequency of the fascicular twitches were less than occurs in the normal subject. However, fasciculations could be induced with an amount of prostigmine (1.0 mgm.) which before thymectomy failed to produce fasciculations in any instance. This observation itself

constitutes objective evidence that, in each patient from whom the thymus was removed, neuromuscular function was altered, for the evidence available indicates that prostigmine fasciculations originate at or near the nerve endings (8).

Action potential. The partial block in neuromuscular transmission which had existed pre-operatively in the patients tested had been decreased by prostigmine, so that the amplitude of the action potential evoked by a single stimulus increased after the administration of prostigmine. This was in direct contrast to the response of a normal subject in whom the amplitude of potential in response to a *single* stimulus was not affected by prostigmine. Post-operatively, in the two patients in whom tests for comparison were available, the change in potential after prostigmine became minimal or insignificant. The patient R. L.

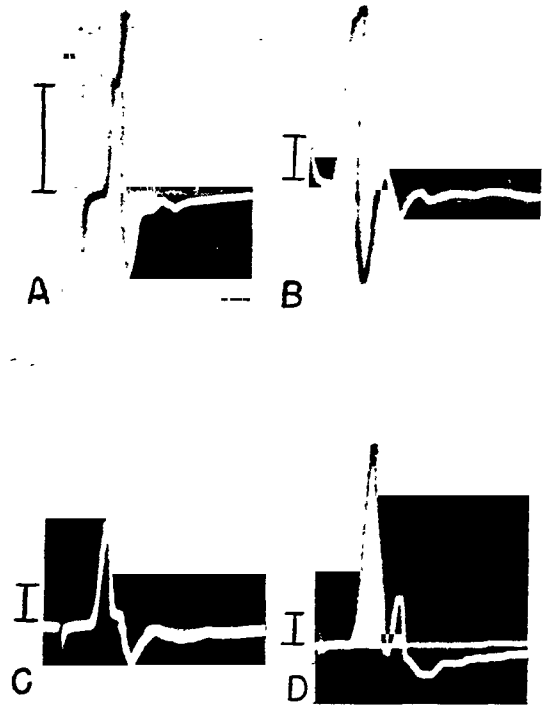


FIG. 5. REPETITIVE RESPONSE TO SINGLE STIMULI AFTER INTRA-ARTERIAL PROSTIGMINE

Patient R. L.: A. Before thymectomy; no repetition. B. After thymectomy a repetitive response appeared.

Patient P. C.: C. Before thymectomy there was no repetitive response. D. Repetitive response developed after thymectomy.

Calibration = 2.0 mV.

had shown a rise in voltage of 34 per cent preoperatively, and five months after thymectomy the rise was less than 2 per cent, which was within the normal variation. The patient P. C. had shown a rise of 19 per cent before thymectomy and 8 per cent after.

Repetitive response. In the normal subject, the response to a single motor nerve stimulus is a single diphasic wave; the intra-arterial injection of prostigmine converts this single wave into a repetitive response, consisting of a series of three or four spikes which fall off rapidly in voltage and duration and which are separated by approximately equal time intervals (3). If the ulnar nerve is stimulated by two maximal stimuli separated by a short interval, the repetitiveness of the second response is greatly diminished until the stimuli have been separated by 48 msec. or more.

In these myasthenic patients before thymectomy we had not been able to detect a repetitive response to single stimuli, although observations had been made after the intra-arterial injection of large doses of prostigmine (2.0 mgm.). However, in three of these patients whose electromyograms were studied, there appeared following thymectomy a repetitive response to single motor nerve stimuli after moderate doses of prostigmine (0.75–1.5 mgm.) (Figure 5). The return of repetitive activity was not complete, for there were only one or two additional spikes in the first response and with the paired stimuli the second response failed to become repetitive. The development of the repetitive response was an additional alteration in neuromuscular function toward a normal pattern.

Neuromuscular transmission. A study of the "two-volley curve" revealed much less depression of neuromuscular transmission after single or multiple responses. The changes observed before the administration of prostigmine have been described above. Before thymectomy, prostigmine always had reduced the neuromuscular junctional depression created by the passage of a single volley; in some instances, normal neuromuscular transmission had been completely restored. After thymectomy, when little or no neuromuscular junctional depression existed in two of the patients (7. s.), the intra-arterial injection of prostigmine produced a slight but definite depression

of conduction after the passage of one volley across the junction. In the patient R. L., for example, five minutes after the intra-arterial injection of prostigmine, when moderate weakness had developed, a train of maximal nerve stimuli evoked responses which fell in amplitude until the fifth response was only 74 per cent of the first (Figures 6 and 7). This progressive depression of the responses after prostigmine is similar to that seen in the normal subject.



FIG. 6. PROSTIGMINE DEPRESSED NEUROMUSCULAR TRANSMISSION AFTER THYMECTOMY

Patient R. L. A. Train of maximal stimuli at 16 msec. intervals before thymectomy; 1.0 mgm. of prostigmine has been given and the responses are equal. B. Same test after thymectomy, at 20 msec. intervals; there is a depression of the successive responses characteristic of the normal. Calibration = 2.0 mV.

The action of acetylcholine

In the normal subject, the intra-arterial injection of 20 to 40 mgm. of acetylcholine produces, among other effects, a transient weakness of the extremity. By contrast, in the myasthenic patient, there

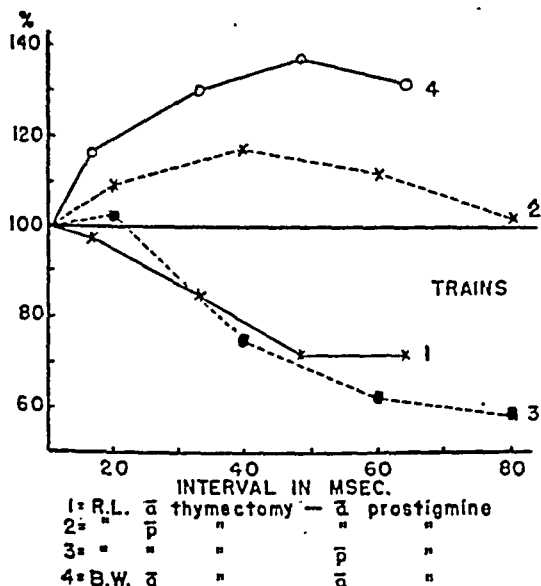


FIG. 7. TRANSMISSION CURVES OF RESPONSES TO TRAINS OF MAXIMAL STIMULI OF THE PATIENT R. L.

Potentials expressed as in Figure 3. 1. Before thymectomy there is a progressive depression; no prostigmine. 2. After operation the responses are facilitated; no prostigmine. 3. After thymectomy prostigmine produces a depression similar to that observed in the normal subject. 4. Patient B. W. (1) showing facilitation during a spontaneous remission.

is a sudden involuntary contraction of the injected muscles. In the patient P. C., the only one in whom we have had the opportunity to restudy the effect of intra-arterial acetylcholine postoperatively, the injection of 20 mgm. of acetylcholine produced a definite brief contraction of the muscles of the injected extremity, but the violence of the contraction was strikingly less than before thymectomy. In addition, as soon as the contraction had relaxed, it was found that the power of the grip was markedly decreased. This transient effect lasted about thirty seconds. It is important to note that this weakness which is characteristic of the normal subject never had been found in this patient before operation.

DISCUSSION

In a previous communication (2) it was suggested that the defect in neuromuscular function which characterizes myasthenia gravis results from a reduction in the amount of transmitter substance, acetylcholine, released at the nerve endings in response to a motor nerve volley. This interpreta-

tion followed a consideration of the evidence summarized below.

(a) Prostigmine repaired the neuromuscular defect occurring in myasthenia gravis and has been shown to protect acetylcholine released at the nerve endings from hydrolysis by cholinesterase.

(b) In the normal subject, prostigmine in high concentration (injected intra-arterially) produced a profound local paresis; in the myasthenic, however, prostigmine increased motor power. Since acetylcholine in sufficiently high concentration depresses neuromuscular function, it was thought likely that the paresis produced in the normal subject results from an accumulation of acetylcholine to a depressant concentration, owing to the anticholinesterase action of prostigmine. The failure of prostigmine to produce weakness in the myasthenic muscle suggests that insufficient acetylcholine is available to accumulate to a depressant concentration (Figure 8).

(c) If the amount of transmitter substance available for release were reduced, it should, in effect, produce functionally a partial "denervation." And this "denervation" might be expected to lower the effector's threshold to the transmitter substance (9). Such sensitization to the transmitter substance, acetylcholine, does occur in the myasthenic muscle; and thus the hypothesis that the transmitter agent has been reduced in quantity gains further support.

This concept of the fundamental defect at the neuromuscular junction emphasizes the relation of the amount of transmitter released to the threshold of muscle excitation by the transmitter. Any factor which increases the amount of transmitter released will improve neuromuscular function in the myasthenic. A secondary effect might be expected to be a corresponding increase in the threshold of the muscle to acetylcholine as the neuromuscular junction approaches normal: a "functional regeneration."

Certain striking similarities between partial curarization and myasthenia gravis suggested that the neuromuscular defect in myasthenia gravis might result from the action of some circulating substance. In the light of known pathological changes occurring in myasthenia gravis, the thymus might be a likely source of this hypothetical agent. This hypothesis has been submitted to experimental

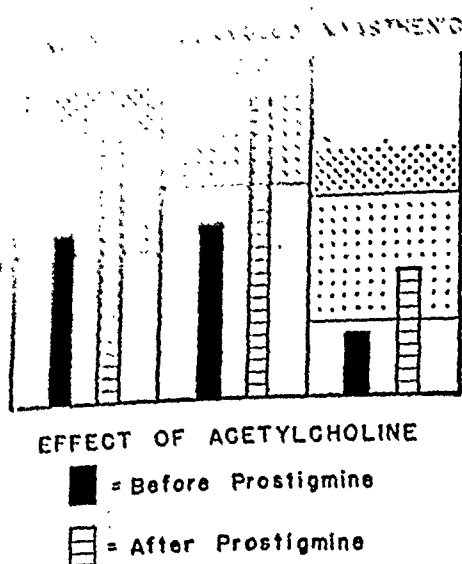


FIG. 8. EFFECT OF ACETYLCHOLINE

The quantum of acetylcholine released by a single stimulus is represented by the solid columns. The effect of this quantum is increased by prostigmine and this is represented by the barred columns. In *normal* muscle, the quantum of acetylcholine released exceeds the excitation threshold and maximal contraction results. When the effect of this quantum is increased by prostigmine until it exceeds the depression threshold, then paralysis results. This depends upon the fact that acetylcholine depresses neuromuscular transmission in higher concentrations. In *curarized* muscle, the quantum of acetylcholine is not altered but the elevation of the excitation threshold renders the normal amount of acetylcholine less effective. Prostigmine increases the effect of this quantum and the contraction range is reached once again. This is the "decurarizing" effect of prostigmine. In *myasthenic* muscle, the excitation threshold is lowered ("functional" partial denervation) but the quantum of acetylcholine is reduced to an even greater degree ("inadequate" acetylcholine release); submaximal response results. Prostigmine enhances the effect of this "inadequate" quantum and, as it exceeds the lowered excitation threshold, improved transmission results.

proof (5), and the results which have been described here, indicate that the thymus plays an important but as yet unidentified role in the pathogenesis of myasthenia gravis. An analysis of this role is being undertaken.

The study of neuromuscular function has indicated that after thymectomy a marked alteration toward a normal pattern of response is observed in patients who exhibited severe myasthenia. There is a marked improvement. There is a marked improvement in the development of local facilitation. Intra-arterial injection of curare into the muscle action potential, in response to a motor nerve stimulus, increased the

that more muscle fibers were excited; the characteristic depression of neuromuscular function following activity at the junction had been eliminated or greatly diminished; the response to a single stimulus after the injection of prostigmine became repetitive as in the normal subject; in two subjects, a normal depression of neuromuscular function developed after the intra-arterial injection of prostigmine; and in one subject, the muscle threshold to acetylcholine injected into the brachial artery rose.

The phenomenon of facilitation which developed in patient R. L. after thymectomy is similar to that observed in another myasthenic patient B. W. (1) during a spontaneous remission. It has not been observed in any other subject, either normal or myasthenic. There are few experimental data which might furnish a clear explanation for the appearance of this change in neuromuscular function, but a consideration of the facts available, suggests a possible explanation which is consistent with the concept of myasthenia gravis proposed before (2).

Brown (10) confirmed the original observations of Bremer and Homès (11) on neuromuscular function in partially curarized preparations, and showed again that the second response to a rapid train of maximal motor nerve stimuli gained in amplitude (facilitation), and then the subsequent responses lost amplitude rapidly until they became constant at a greatly reduced level. Brown suggested that this long depression is due to insufficiently rapid replacement of acetylcholine, to which curare has raised the threshold of the muscle fibers. He stated that the augmentation of the first response occurring during the first 50 of the train of stimuli is altered from a separate process of facilitation. His observations may be interpreted in terms of the amount of acetylcholine released by a single stimulus in a train of stimuli. The rate of its synthesis is

equally important. graphically the released by the facilitation. In the muscle fibers acetylcholine

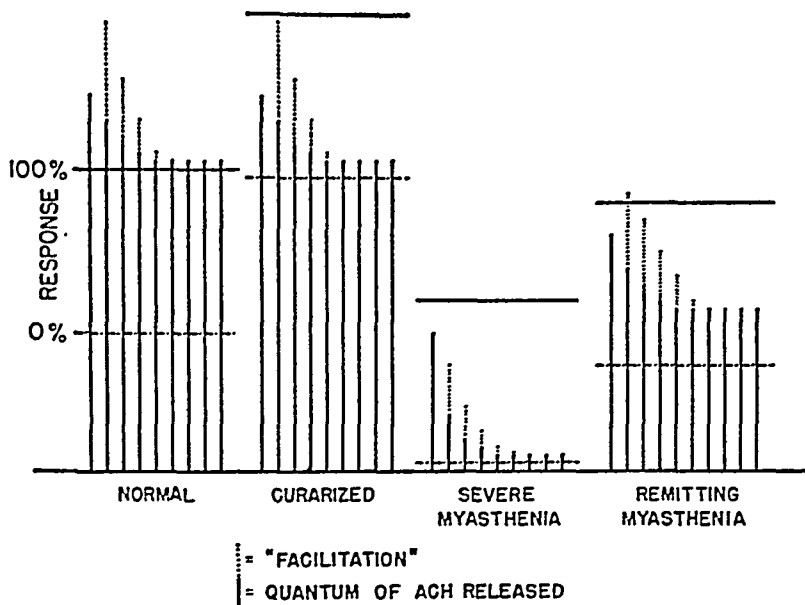


FIG. 9. THEORETICAL DIAGRAM RELATING THE QUANTA OF TRANSMITTER SUBSTANCE RELEASED TO THE APPEARANCE OF FACILITATION

For description see text.

released has reached its minimum, because this minimum still exceeds the threshold for response of the least excitable fibers. Thus any change in amount of transmitter occurring above this threshold will not be detected. When the muscle has been partially curarized, however, the threshold at which every fiber responds is higher. Consequently the variations in amount of transmitter released, and in the facilitation process, will appear as variations in the submaximal response of the muscle as a whole. In the state of severe myasthenia, the hypothetical fall in acetylcholine output is represented by the shorter solid lines which diminish very sharply. The concomitant lowering of muscle threshold to acetylcholine is represented by the lower threshold for total fiber response. The facilitation process may operate here as well as in the normal or curarized muscle, but the abrupt decline in the amount of acetylcholine released obscures the facilitation, so that the responses still decline rapidly. During a remission, whether spontaneous or induced, the threshold for total fiber response rises toward normal and the amount of available transmitter is assumed to increase. This would constitute a transition stage between severe myasthenia and normal function; in this stage, the relation of muscle threshold to the amount of transmitter re-

leased would be similar to that occurring in the partially curarized muscle. Under these circumstances, the previously obscured process of facilitation would become apparent.

SUMMARY

1. Five patients with severe myasthenia gravis have been restudied up to five months after total extirpation of the thymus. Three have shown a great degree of clinical improvement.

2. Electromyographic studies demonstrated that (a) a larger number of muscle fibers responded to a maximal motor nerve stimulus, and (b) there was greater efficiency in the transmission of pairs and trains of maximal motor nerve stimuli across the neuromuscular junction.

3. The intra-arterial injection of prostigmine, in contrast to its effect before thymectomy, now produced (a) local fascicular twitching, (b) repetitive response to a single stimulus, (c) normal local paresis and a depression of neuromuscular transmission in two of the patients, and (d) greater effect of prostigmine in the third.

4. In one patient, the intra-arterial injection of acetylcholine produced less contraction than before thymectomy and the contraction was followed by transient weakness.

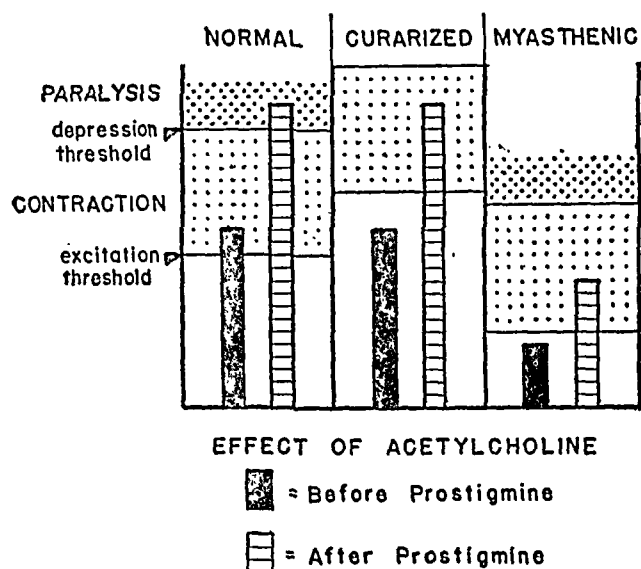


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proof (5), and the results which have been described here, indicate that the thymus plays an important but as yet unidentified role in the pathogenesis of myasthenia gravis. An analysis of this role is being undertaken.

The study of neuromuscular function has indicated that after thymectomy profound alterations toward a normal pattern occurred in the three patients who exhibited such extraordinary clinical improvement. There has been recorded the development of local fasciculations following the intra-arterial injection of prostigmine; the muscle action potential, in response to a maximal motor nerve stimulus, increased enormously, indicating

that more muscle fibers were excited; the characteristic depression of neuromuscular function following activity at the junction had been eliminated or greatly diminished; the response to a single stimulus after the injection of prostigmine became repetitive as in the normal subject; in two subjects, a normal depression of neuromuscular function developed after the intra-arterial injection of prostigmine; and in one subject, the muscle threshold to acetylcholine injected into the brachial artery rose.

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This theory is applied to myasthenia graphically in Figure 9. The decline in acetylcholine released toward a constant output is represented by the solid lines. The brief superimposed facilitation process is indicated by the broken lines. In the normal state, all of the innervated muscle fibers respond even when the amount of acetylcholine

EFFECTS PRODUCED BY THE INTRAVENOUS INJECTION IN MAN OF A TOXIC ANTIGENIC MATERIAL DERIVED FROM *EBERTHELLA TYPHOSA*: CLINICAL, HEMATOLOGICAL, CHEMICAL AND SEROLOGICAL STUDIES

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The preparation of purified antigenic materials from cultures of *Eberthella typhosa* (1, 2) has demonstrated that substances of relative purity, and high antigenic and toxic activity, may be isolated for study. In investigations utilizing a synthetic medium for the culture of *E. typhosa* (3), which eliminates the possibility of non-specific contaminating materials (derived from the culture medium), a toxic, antigenic material has been isolated which has been extensively studied. This material acts as a complete antigen, giving rise to the formation of agglutinins (4), precipitins (4), bactericidal antibodies (5), and mouse-protective antibody (6). Possessing marked toxicity, it kills mice, rabbits, and guinea pigs with relatively small doses (4). A study of the tissues obtained from animals receiving intravascular injections of the material has shown pathological changes involving the heart muscle, lung, liver, spleen, bone marrow, and vascular bed (7). On intravenous injection in rabbits, the material induces a pronounced leukopenia and a marked temperature rise (4). Because of these properties it was desired to determine, insofar as possible, whether the material might exhibit similar effects following intravenous injection into patients for the purpose of inducing therapeutic febrile reactions.

MATERIALS AND METHODS

Preparation of antigen

Following the technique previously described (3, 4) involving repeated precipitations with alcohol and resuspension in water, an antigenic material was obtained from cultures of *E. typhosa* grown in a liquid medium which consisted only of ingredients removable by dialysis (3). The material which contained the O and Vi antigens possessed all the properties exhibited by that previously studied (4). The stock solution of antigen was made up

in distilled water and stored in the refrigerator for over a year. During this time no loss of activity was observed. From this stock solution containing 1 mgm. per cc. of the antigen, successive dilutions were prepared in physiologic saline for intravenous administration.

Methods of administration

The patients selected for this study represented 11 cases of asymptomatic neurosyphilis, and 1 normal person. The group included 7 males and 5 females. The injections were administered intravenously into the median basilic vein. Observations on temperature, pulse, and respiration were recorded at hourly intervals.

Hematological determinations

At hourly intervals after the injections, total and differential leukocyte counts were made on capillary blood obtained from a finger. At certain intervals, erythrocyte counts and hemoglobin (Sahli) determinations were carried out. On several patients, repeated determinations of the sedimentation rate were made, using 15 cc. of blood to which was added 0.2 cc. of 20 per cent potassium oxalate. This blood was placed in a Cutler tube and the rate of sedimentation recorded. The tube was then placed in the centrifuge and spun at 2500 r.p.m. for 45 minutes. The volume of packed cells was then measured with calipers and expressed as a percentage of the total volume in the tube.

Chemical determinations

Hourly measurements of certain constituents of the blood were made following the administration of various amounts of the antigen, using oxalated blood prepared by adding 0.2 cc. of 20 per cent potassium oxalate to 15 cc. of blood. Total proteins were determined by the falling drop method (8), urea nitrogen determinations were obtained by the method of Karr (9), and creatinine by the technique of Folin and Wu (10). Blood glucose values were obtained using the method of Folin and Wu (11), chlorides by that of Whitehorn (12), and the carbon dioxide combining power of the plasma by the technique of Van Slyke and Cullen (13). Throughout the 12 hours of experiment, the patients received only water,

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5. The evidence indicates that in certain individuals the thymus plays an important role in the pathogenesis of myasthenia gravis. The changes after thymectomy indicate an increase in the amount of transmitter substance available at the neuromuscular junction.

BIBLIOGRAPHY

1. Harvey, A. M., Lilienthal, J. L., Jr., and Talbot, S. A., Observations on the nature of myasthenia gravis. The phenomena of facilitation and depression of neuromuscular transmission. *Bull. Johns Hopkins Hosp.*, 1941, 69, 547.
2. Harvey, A. M., and Lilienthal, J. L., Jr., Observations on the nature of myasthenia gravis. The intra-arterial injection of acetylcholine, prostigmine, and adrenaline. *Bull. Johns Hopkins Hosp.*, 1941, 69, 566.
3. Harvey, A. M., Lilienthal, J. L., Jr., and Talbot, S. A., On the effects of the intra-arterial injection of acetylcholine and prostigmine in normal man. *Bull. Johns Hopkins Hosp.*, 1941, 69, 529.
4. Marshall, W. H., and Talbot, S. A., Multi-channel delay unit. *Rev. Scient. Instruments*, 1940, 11, 287.
5. Blalock, A., Harvey, A. M., Ford, F. R., and Lilienthal, J. L., Jr., Treatment of myasthenia gravis by removal of thymus gland; preliminary report. *J. A. M. A.*, 1941, 117, 1529.
6. Harvey, A. M., and Masland, R. L., Method for study of neuromuscular transmission in human subjects. *Bull. Johns Hopkins Hosp.*, 1941, 68, 81.
7. Harvey, A. M., and Masland, R. L., Electromyogram in myasthenia gravis. *Bull. Johns Hopkins Hosp.*, 1941, 69, 1.
8. Masland, R. L., and Wigton, R. S., Nerve activity accompanying fasciculation produced by prostigmin. *J. Neurophysiol.*, 1940, 3, 269.
9. Cannon, W. B., Law of denervation. *Am. J. M. Sc.*, 1939, 198, 737.
10. Brown, G. L., The effect of small doses of curarine on neuro-muscular conduction. *J. Physiol.*, 1938, 92, 23P.
11. Bremer, F., and Homès, G., Une theorie de la sommation d'influx nerveux. *Arch. Internat. de Physiol.*, 1932, 35, 39.

disturbing. After several injections, in each of which the dosage was doubled, it became possible to increase subsequent doses fivefold without causing severe reactions. An interval of at least one day was allowed between each injection. Some patients became capable of tolerating as much as

0.5 mgm. in a single injection, following gradual increase of dosage over a period of several weeks. This increase proved necessary to obtain adequate febrile responses, because the patients developed a tolerance to the antigen after repeated injections of amounts which previously had been

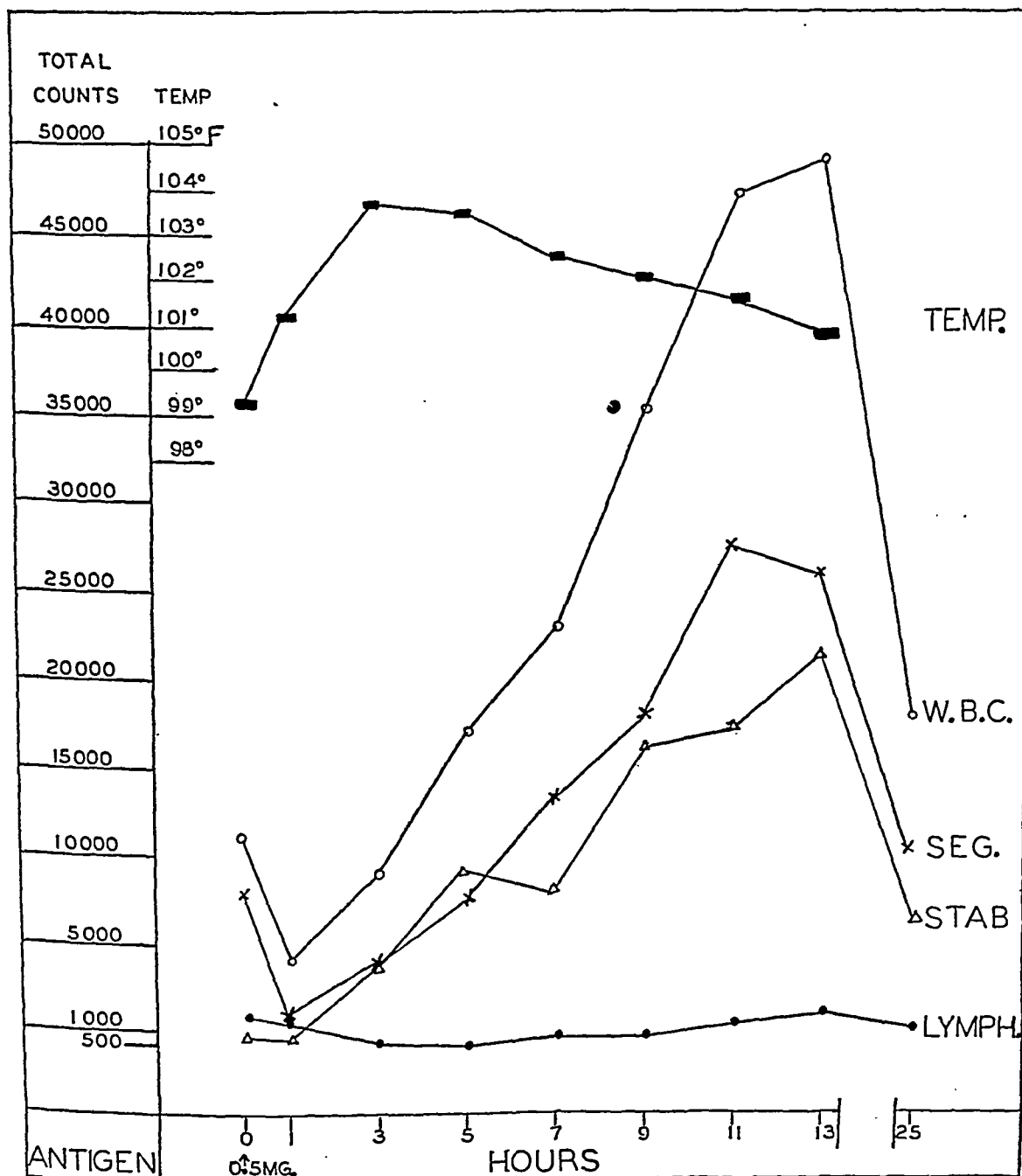


FIG. 1. TYPICAL FEBRILE AND LEUKOCYTE RESPONSES FOLLOWING INJECTION OF ANTIGEN

Agglutinins

At certain periods, serum was obtained from the patients just before the subsequent injection, and its titer of antibody measured by agglutination, precipitation and bactericidal tests. Alcohol treated bacterial suspensions of *E. typhosa* strain 0901, and serial dilutions of the serum in a macroscopic test, were used to measure the titer of 0 agglutinins. H agglutinins were assayed by means of a suspension of the H901 strain of *E. typhosa* to which 0.2 per cent formalin was added. A rough strain of *E. typhosa* containing the Vi antigen was used in a saline suspension to test for the presence of Vi agglutinins. These tests were read after incubation for 2 hours at 37° C. and after remaining overnight in the refrigerator. The titers were recorded as the highest dilution producing a +++ reaction in a series graded from + to ++++.

Precipitins

These tests were carried out by the ring technic. Undiluted serum was placed in a series of tubes over which were layered dilutions of the antigen in saline. The tests were read after 1 hour at room temperature (20 to 24° C.).

Bactericidal antibodies

The determinations of bactericidal antibody were made by means of a technique previously described (5) in which measured amounts of the serum dilutions, diluted broth culture of the strain of *E. typhosa* from which the antigen was prepared, and 0.25 cc. of diluted guinea pig serum were added to a series of small Pyrex glass tubes. After these tubes were sealed in an oxygen-gas flame, they were placed at 37° C. in a rotating device which turned at the rate of 7 to 8 revolutions per hour. Twenty-four hours later the contents of the tubes were distributed over the surfaces of infusion agar plates, which were then incubated at 37° C. for 48 hours. The titer was taken as the highest dilution which killed all of the organisms added to the tube. In each experiment, suitable controls were included to reveal any inhibition of bacterial growth alone attributable to the antiserum or the diluted guinea pig serum. For each patient, the bactericidal tests performed on the 2 serum samples were carried out simultaneously to avoid differences due to variations in the materials used, *i.e.*, complement and bacterial culture.

EXPERIMENTAL

1. Determination of the effective dose and subsequent rejections

Because previous experiments with animals had shown that the antigen was toxic in very small amounts (4), the small initial dose of 0.0001 mgm. of antigen in 1 cc. of saline was administered. Following this injection, a rise in rectal temperature to levels varying from 100.2 to 101.8° F. oc-

TABLE I

Temperature and agglutinin response to initial and subsequent antigen injections

Patient	Date	Dose mgm.	Maximum tempera- ture— rectal	0 agglu- tinin titer dilution of serum
1	January 28, 1941	0.0001	100.2°F.	
2	October 15, 1940	0.0001	100.2	
	October 22, 1940	0.004	101.8	
	October 23, 1940	0.01	104.4	
	October 25, 1940	0.01	102.4	
	October 26, 1940	0.01	102	
	October 28, 1940	0.01	102.2	
	October 29, 1940	0.01	101.4	1 : 8192
3	December 9, 1940	0.0001	100.6	1 : 16
	December 20, 1940	0.05	103.8	1 : 32
	December 23, 1940	0.1	106.6	1 : 256
	December 26, 1940	0.1	103	1 : 256
	December 27, 1940	0.1	102.6	1 : 512
4	November 6, 1940	0.0001	101.6	0
	November 15, 1940	0.04	103.4	1 : 4096
	November 17, 1940	0.1	105.2	1 : 8192
	November 19, 1940	0.1	104.4	
	November 21, 1940	0.3	105.8	1 : 4096
	November 23, 1940	0.3	104.6	
5	February 4, 1941	0.0002	100.2	1 : 20
6	November 25, 1940	0.0002	100.4	1 : 8
	November 26, 1940	0.001	103.0	
	November 28, 1940	0.005	103.2	
	November 30, 1940	0.01	104.2	1 : 128
	December 3, 1940	0.05	101.4	1 : 2048
	December 6, 1940	0.1	102.6	1 : 8192
	March 7, 1941	0.01	102.8	
	October 17, 1941	0.03	103	
	January 28, 1942	0.05	105	1 : 512
7	June 18, 1941	0.0002	101	0
	June 20, 1941	0.02	105	0
	June 23, 1941	0.02	103	1 : 512
	June 25, 1941	0.1	102.4	1 : 2048
	July 1, 1941	0.5	105.2	1 : 2048
	July 3, 1941	0.5	101.6	1 : 4096
8	April 17, 1941	0.0002	101.8	0
9	June 25, 1941	0.0005	101.2	1 : 8
10	November 3, 1940	0.001	102.6	0
11	January 22, 1941	0.001	101	0

curred. Doubling this dose on the following injection caused a rise to values of 100.2° to 101.8° F. One patient was given 0.005 mgm. and 2 were given 0.01 mgm. as the initial dose (Table I). The temperatures rose to 101° F. and 102.6° F., respectively.

From this data it is evident that an initial dose of less than 0.001 mgm. proved quite safe and that the systemic reactions which occurred were not

especially the stab and segmented forms. An occasional metamyelocyte was seen.

During the first hour after the injection, usually the period of leukopenia, the stab cells showed a slight increase, with a subsequent sharp rise during the second hour to a level 2 to 4 times that of the first hour (Table III and Figure 1). This level was maintained and was even increased as long as the leukocytosis persisted, with a return towards the original level as the total leukocyte count fell to normal values. If the patient had received a series of consecutive injections, the stab cell count was high originally and tended to remain above its initial level, even though the total leukocyte count was approaching pre-injection values, as is shown in the case of patient 7 (Table III). With the larger doses of antigen, the reaction was accompanied with a more marked leukocytosis and a correspondingly higher stab cell count.

The segmented cells showed a definite decrease during the first hour which was roughly proportional to the degree of leukopenia (Figure 1).

This was followed by an hourly increase which more or less paralleled the total leukocyte count, with the highest values for segmented cells being attained in 8 to 15 hours during the height of the leukocytosis. The restitution to normal levels was more rapid than that in the case of the stab cells, but was influenced by the same factors.

Both stab and segmented forms revealed toxic granulation of the cytoplasm which was more marked with larger amounts of the antigen. The preponderance of the stab cells was similar to that seen in the blood picture of patients with typhoid fever (14).

During the first hour (leukopenic phase) there was little change in the number of lymphocytes, with a resultant relative increase; this was in marked contrast to the changes described in the numbers of stab and segmented leukocytes. However, there was a sudden decrease between the 3rd to 6th hours with a subsequent gradual increase reaching normal or somewhat greater values during the phase of leukocytosis as seen in Table III and Figure 1.

TABLE III

Differential leukocytic count. Changes in metamyelocytes, stab cells, segmented cells and lymphocytes

Hours	Total white count	Metamyelocytes		Stab cells		Segmented cells		Lymphocytes	
			Number		Number		Number		Number
<i>Patient 1</i>	<i>per mm.³</i>	<i>per cent</i>	<i>per mm.³</i>	<i>per cent</i>	<i>per mm.³</i>	<i>per cent</i>	<i>per mm.³</i>	<i>per cent</i>	<i>per mm.³</i>
0	6500			6	390	43	2795	49	3185
				0.12 mgm. antigen intravenously					
1	5600			13	728	33	1848	54	3024
2	7900	1	79	37	2923	48	3792	14	1106
3	6300			47	2961	41	2583	12	756
4	7900			37	2923	56	4424	6	474
5	8200			34	2788	58	4756	8	656
6	9900			42	4158	52	5148	6	594
7	13700			30	4110	61	8357	8	1096
9	25500			39	9945	54	13770	6	1530
11	27600			10	2760	78	21528	12	3312
13	24900			13	3237	76	18924	11	2739
15	21400			11	2354	69	14766	20	4280
28	11500			5	550	77	8470	18	1980
30	9800			8	784	65	6370	27	2646
<i>Patient 7</i>									
0	5400			22	1188	47	2538	27	1458
				0.02 mgm. antigen intravenously					
2	6400			39	2496	39	2496	17	1008
4	11800	1	118	48	5664	39	4602	12	1416
6	11000	4	440	40	4400	53	5830	3	330
8	24800			49	12152	44	10912	7	1736
12	29800	1	298	55	16390	35	10430	8	2384
14	28100			45	12645	31	8711	21	5901
24	10700	1	107	47	5029	37	3959	13	1391
30	10700			36	3852	35	3745	23	2461
35	13800			33	4554	50	6900	17	2346

TABLE II
Changes in leukocyte count following injection of antigen
Patient 8

Date	Dose	Leukocyte count at hourly intervals following injection				Maximum leukocyte count		Restoration to normal leukocyte count	
							Time after injection		Time after injection
	mgm.	0	per mm. ³		3	per mm. ³	hours	per mm. ³	hours
April 17, 1941	0.0002	7200	4200	4000	4400	13500	6	8000	12.5
April 18, 1941	0.0006	4200	2000	2600	7600	15000	7	8000	14
April 23, 1941	0.001	5400				10500	4.5	9500	6.5
April 25, 1941	0.006	4800	2200	6600	7200	20500	10	5600	24
April 30, 1941	0.03	6000	2200	7000	6800	37500	12	10000	28
May 2, 1941	0.03	7200		3600	8000	32000	8	10400	24

adequate. The data in Table I illustrate this decreasing response which was particularly striking in patients 3 and 7. Tolerance developed upon repeated injections regardless of the increase of the amount administered in a single injection. The data on patient 6 indicates, however, that the refractory state was maintained only by continued injections. The reactions to reinjection at considerable intervals of time in this patient also suggest that the antibody titer is not closely correlated with this resistant state, since the titer remained considerable at a time when the patient developed febrile reactions of 103° and 105° F., following injections (Table I).

2. Systemic reactions following injections

When an effective dose was administered, the symptoms elicited were quite uniform. Within 30 to 40 minutes following the injection, the patient experienced a chilly sensation which culminated in a definite rigor of varying intensity which lasted from 25 to 35 minutes. This was followed by generalized aching sensations, which at times were accompanied by localization of pain in some part of the body such as the head, legs, or back. Nausea and occasional vomiting also accompanied these symptoms. Within the first hour, the temperature rose above 100° F., reaching its fastigium in 2 to 3 hours and falling to 100° F., or less, within 7 to 8 hours. The pulse and respiration showed a corresponding rise. A typical temperature curve is presented in Figure 1.

Perspiration made its appearance during the height of the fever and continued for 2 or 3 hours.

This was followed by a feeling of fatigue, exhaustion and a diminution in the intensity of the other symptoms.

3. Alterations in the cellular components of the blood following injection

a. Total leukocyte counts. The total leukocyte count followed a characteristic pattern in part dependent on the amount of antigen administered. With an effective dose, a definite fall in the leukocyte count occurred during the first 2 hours. The degree of leukopenia was proportional to the amount of antigen injected. Again with repeated injections, as in the case of the febrile reaction, larger amounts of antigen were required to produce a leukopenia of the same degree. A representative protocol is presented in Table II.

Following the leukopenia, a gradual increase in the number of leukocytes occurred until the count returned to its normal level after 3 to 4 hours. Subsequently, a leukocytosis was observed within 9 to 12 hours (Figure 1). The count then decreased until the normal level was again reached. The time required for the development of leukocytosis and subsequent return to the normal state appeared to be proportional to the dosage. Total white counts of 30,000 per mm.³, or higher, were not uncommon, and some values as high as 67,000 per mm.³ were obtained. When such pronounced leukocytosis occurred, the return to a normal level required a longer time interval (Table II).

b. Differential leukocyte counts. The cellular responses described were largely due to changes in the numbers of polymorphonuclear leukocytes,

the median basilic vein, on several patients, which yielded values with variations within experimental error.

c. Hemoglobin and erythrocytes. The erythrocytes and hemoglobin failed to show any definite change following a single injection, but after repeated doses, however, several of the patients showed a definite reduction in both hemoglobin and red cells. Such changes have been described in typhoid fever by Holmes (15). In addition to the other determinations, the volume of packed cells was followed throughout the paroxysm in order to detect any changes due to fluid loss produced by the perspiration and vomiting. No significant alterations were observed. However, patients were allowed to partake of fluids at will, though the fluid intake was usually minimal because of the nausea so frequently present. The sedimentation rate of the erythrocytes was followed during the period of observation of the patients and particularly during the administration of the antigen. Following the initial administration of antigen, the sedimentation rate rose appreciably. This elevation was maintained and often increased by subsequent administration. Starting with an elevated rate, little or no variation was noted in the rate, by hourly determinations during the paroxysm. Data typical of the series are presented in Table IV.

4. Chemical changes in the blood

In summarizing the changes in the chemical constituents of the blood, it may be said that there was a slight to moderate immediate reduction in the values of blood glucose and a subsequent rise in 5 to 7 hours to levels above the initial values, in most cases. The patients showed some reduction in the values for blood chlorides and in the carbon dioxide combining power of the plasma. These data are summarized in Table V. There were no significant variations in the values for total protein, urea nitrogen, or creatinine.

5. Immunologic responses to the injections

O agglutinins were demonstrable as early as the 5th day after the initial injection, and reached their maximum titer at about the end of the 2nd week (Figure 2). This level was approximately

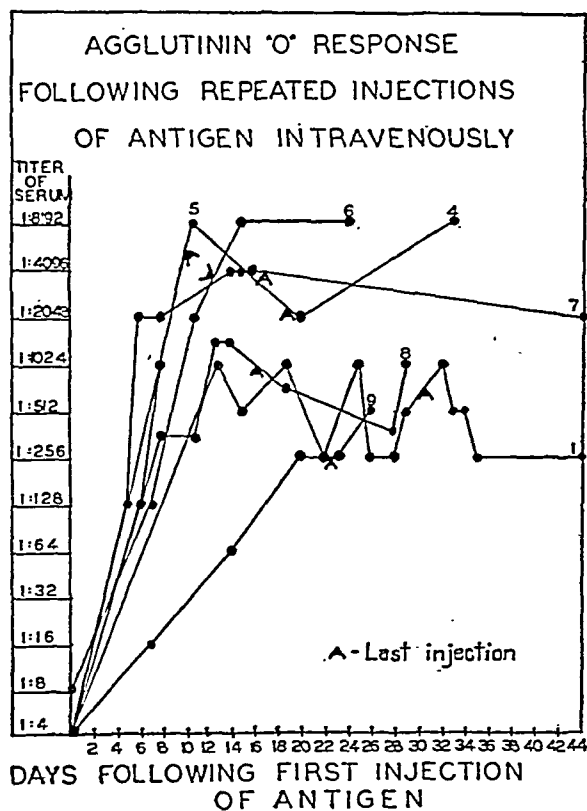


FIG. 2. AGGLUTININ TITERS IN PATIENTS FOLLOWING THE INJECTION OF ANTIGEN

maintained for considerable periods of time, as demonstrated in patients 1, 6, and 7. In several instances in which the sera of patients were re-tested 6 weeks after the last injection of antigen, only a small decrease in the titer was noted. Vi agglutinins were present in most instances. H agglutinins were not detected.

The precipitin titers were performed at less frequent intervals but bore evidence to a good antigenic response by attaining values which ranged from 1:10,000 and 1:100,000 dilutions of antigen (Table VI).

Since previous experiments (5) had indicated that the intravenous injection of the antigen resulted in the formation of bactericidal antibodies for *E. typhosa*, the sera of patients were tested for their presence. Progressive development of high titers was demonstrated (Table VI). Figure 3 presents a correlation of these various antibody titrations with the dosage of antigen administered in case 7. Table VI summarizes antibody titers and total dosage of antigen administered.

TABLE IV

Sedimentation rate following the injection of the antigen

Patient	Date	Dose	Injection number	Sedimentation rate of blood drawn at hourly intervals							
				0	1	2	3	4	5	6	7
		mgm.		mm. per hour							
1	February 11, 1941	0.12	7	19	19.5	20	18	20.5	20	20	21
	February 18, 1941	0.5	9	23	25	23	22	22.5	24	23.5	19.5
	February 25, 1941	0.6	11	26.5	28	29	27.5	28	27	26	28
7	June 20, 1941	0.02	3	21	18	17.5	16.5	17.5	17.5	18.5	18
	July 1, 1941	0.5	6	21	21	18	19	17	17	16	18
12	January 22, 1942	0	0	2.0							
	January 24, 1942	0.001	1	15							
	January 27, 1942	0.01	2	17							
	February 2, 1942	0.2	3	15							
	February 13, 1942	0.8	10	15							

The monocytes and basophiles showed no outstanding changes. Eosinophiles were constantly absent during the phase of the leukocytosis.

To check the determinations, simultaneous leukocyte counts were made on blood obtained from the finger and oxalated blood obtained from

TABLE V

Fasting blood glucose, chlorides, and carbon dioxide combining power of the plasma, following the administration of antigen

Patient	Date	Antigen	Glucose at hourly intervals							
			0	1	2	3	4	5	6	7
		mgm.	mgm. per 100 cc. of blood							
1	February 11, 1941	0.12	90	83	90	95	95	95	95	100
	February 18, 1941	0.5	100	83	95	95	100	95	95	95
	February 25, 1941	0.6	100	80	83	86	100	100	133	100
7	June 20, 1941	0.02	95	80	68	83	90	100	100	111
	July 1, 1941	0.5	86	73	36	36	34	40	50	60
8	April 25, 1941	0.006	90	86	95	111	118	118	125	118
	April 30, 1941	0.03	95	80	95	111	111	118	111	111
9	July 18, 1941	0.1	80	77	68	68	80	77	77	100
			Sodium chloride							
			mgm. per 100 cc. of blood							
1	February 11, 1941	0.12	492	463	454	471	500	394	437	496
	February 18, 1941	0.5	484	474	412	446	480	480	446	428
	February 25, 1941	0.6	528	462	462	462	363	412		445
7	June 20, 1941	0.02	528	495	495	544	511	478	478	495
	July 1, 1941	0.5	495	412	472	511	462	445	445	495
8	April 18, 1941	0.0006	493	435	464	551	435	450	435	449
	April 25, 1941	0.006	560	495	528	478	511	495	495	495
9	April 30, 1941	0.03	560	511	528	495	495	495	495	478
	July 18, 1941	0.1	478	495	478	462	445	462	462	429
			Carbon dioxide combining power							
			cc. CO ₂ per 100 cc. of plasma							
1	February 25, 1941	0.6	46.6			44.7				39.0
7	June 20, 1941	0.02	55.3				44.8			44.7
	July 1, 1941	0.5	46.6			33.4				37.2
8	April 18, 1941	0.0006	46.6			40.9				37.2
	April 25, 1941	0.006	44.7			46.6				39.0
9	April 30, 1941	0.03	44.7				46.6			40.9
	July 18, 1941	0.1	46.6			45.7				44.7

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In summarizing the changes in the chemical constituents of the blood, it may be said that there was a slight to moderate immediate reduction in the values of blood glucose and a subsequent rise in 5 to 7 hours to levels above the initial values, in most cases. The patients showed some reduction in the values for blood chlorides and in the carbon dioxide combining power of the plasma. These data are summarized in Table V. There were no significant variations in the values for total protein, urea nitrogen, or creatinine.

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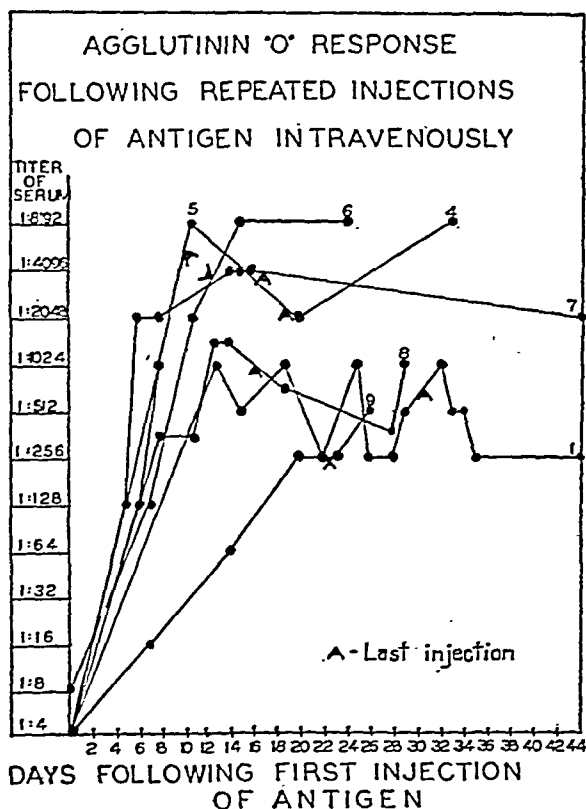


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TABLE VI
Bactericidal, precipitin, and agglutinin titers, following a series of injections of the antigen

Bactericidal, precipitin, and agglutinin titers, following a series of injections of

Patient	Date blood was taken	Bactericidal titer	Precipitin titer	Agglutinin titer		Days between 1st injection and 1st titer	Days between 1st injection and 2nd titer	Accumulative dose of antigen at	
		Dilution of serum	Dilution of antigen	Dilution of serum				1st titer	2nd titer
				0	VI				
1	January 30, 1941 February 25, 1941	1 : 40 1 : 20480	1 : 100 1 : 100000	1 : 4 1 : 2048	0 1 : 16	2	27	mgm. 0.0006	mgm. 1.6316
2	November 6, 1940 April 28, 1941	1 : 20480 1 : 5120	1 : 100000 1 : 10000	1 : 4096 1 : 1024	1 : 8 1 : 2	23	195	0.8076	0.8076
3	December 9, 1940 January 18, 1941	1 : 64 1 : 1024	1 : 10 1 : 10000	1 : 2 1 : 512	0 1 : 16	0	40	0.0000	0.6665
4	November 6, 1940 December 9, 1940	0 1 : 10240	0 1 : 100000	0 1 : 4096	0 1 : 8	0	33	0.0000	0.9033
5	February 6, 1941 March 4, 1941	1 : 128 1 : 40960	1 : 10 1 : 100000	1 : 20 1 : 8192	0 1 : 16	2	28	0.0002	0.0682
7	June 20, 1941 July 3, 1941	1 : 2 1 : 20480	0 1 : 100000	0 1 : 4096	0 1 : 64	2	15	0.0022	1.1122
8	April 18, 1941 May 29, 1941	1 : 128 1 : 20480	1 : 10 1 : 100000	0 1 : 1024	0 0	1	42	0.0002	0.0876
9	June 25, 1941 July 21, 1941	1 : 160 1 : 10240	1 : 100 1 : 100000	1 : 8 1 : 2048	0 0	0	26	0.0005	0.4530

it is released during the growth of the typhoid bacillus again suggests that it might be related to the development of certain clinical manifestations of typhoid fever in patients, as was earlier postulated (4) on the basis of the animal experiments.

The most striking changes noted in the studies of the blood were the alterations in the total and differential leukocyte counts. The leukopenia which developed in patients following adequate dosage was due almost entirely to a decrease in the number of polymorphonuclear leukocytes (Figure 1). The subsequent return of the leukocyte count to normal levels and the later development of a leukocytosis was due largely to a release of young polymorphonuclear leukocytes from the bone marrow.

The mechanism of the production of the leukopenia following the intravenous injection of typhoid bacilli in animals and man is a controversial matter. The conflicting theories and their supporting evidence have been summarized by Garrey and Bryan (18) who discuss the possibility that the leukocytes are trapped in the vascular bed of internal organs, as opposed to the view that the

leukopenia may be due to the destruction of these cells. The results of several investigations (18) give evidence for such accumulation of polymorphonuclear leukocytes within the vessels of the lung, spleen, and other organs of animals, following the intravenous injection of typhoid bacilli or toxic filtrates derived from cultures of *E. typhosa*. These observations are in contrast to the earlier findings of Pepper and Miller (19) who failed to show such accumulations. Pepper and Miller also stated that Arneith differential counts made during the leukocytosis, which superseded the leukopenia, showed such a great increase in the numbers of young polymorphonuclear leukocytes as to suggest that at least part of the mature leukocytes had not returned to the circulation. A like situation was reported in the experiments of Cowie and Calhoun (16) and Perera (17) who studied the changes in the leukocyte count following the intravenous injection of typhoid vaccine into patients. Both of these authors report a "shift to the left" in the differential polymorphonuclear leukocyte counts during the leukocytosis, which suggests that the leukocytosis is due to a

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release of young cells from the bone marrow. The striking increase in the numbers of stab forms of polymorphonuclear leukocytes, demonstrated in these experiments at a time when the leukocyte count had increased only to normal levels, indicates that this return to normal was not due to a release of adult cells that had been trapped in internal organs, but rather to an increased activity of the bone marrow, comparable to that previously described in animals that received injections of the antigenic material (7). Moreover, the recent experiments of Dennis and Senekjian (20), which show that a similar antigenic material isolated from cultures of *E. typhosa* has a selective destructive action on polymorphonuclear leukocytes when added to human blood *in vitro*, further support the hypothesis that these substances produce the leukopenia, at least in part, by the destruction of polymorphonuclear leukocytes.

The blood picture following the injection of this antigenic material is similar to that described by Schilling (14) in patients with typhoid fever, with its neutropenia, increased number of stab cells, and absence of eosinophiles.

The failure of the injection of this material to affect appreciably most of the chemical constituents of the blood is in agreement with the data of Perera (17). The tendency for the blood sugar to rise is similar to the observations by Delafield (21) that somatic antigens of the *Salmonella* group produce a hyperglycemia in animals.

The immunologic responses of the patients to the injection of the antigen reveal that it has high potency as an antigenic agent and suggests that it might be of use in immunization. Studies are now in progress on a group of volunteers to elucidate this property, and preliminary results (6) indicate that the material will prove of value. Again, as in the earlier experiments utilizing rabbits (4), it is suggested that a high titer of circulating antibody is not correlated with resistance to the toxic effect of the antigen, as larger doses produced toxic effects in the presence of very high antibody titers (Table I). A certain degree of resistance to the toxicity of the antigen developed following consecutive administration but injections made several months later, as in the case of patient 6, produced febrile reactions of considerable intensity at a time when the blood serum showed a significant titer of agglutinins. The

significance of this observation that circulating antibody cannot be correlated with the resistance to toxicity has been previously discussed in detail with regard to the experiments utilizing rabbits (4). These observations may, in part at least, account for the seemingly paradoxical fact that symptoms of toxemia in typhoid fever persist in the presence of circulating antibody whether this be naturally present or introduced through the administration of antiserum.

SUMMARY

1. An antigenic material prepared from cultures of *E. typhosa* grown in a synthetic medium produced chills, fever, perspiration, and muscular aching, following the intravenous injection of minute amounts in man. The severity of the reaction was dependent on the amount of material administered. With consecutive injections, larger doses were required to produce similar systemic reactions, indicating a tolerance to its toxicity.

2. The injection of the material was followed by the development of a leukopenia which was almost entirely due to a decrease in the numbers of polymorphonuclear leukocytes in the capillary blood. This leukopenia was followed by a return of the leukocytes to normal levels, and by a subsequent leukocytosis in which the number of stab cells was markedly increased.

3. No marked changes were observed in determinations of total protein, urea nitrogen, creatinine, chloride, glucose, and carbon dioxide combining power of the blood, during and in the several hours following the reaction. The sedimentation rate of the erythrocytes rose after injections.

4. Serological tests, following repeated injections, revealed the production of high titers of agglutinins, precipitins, and bactericidal antibodies, which then remained at high levels for considerable periods of time. The titer of the circulating antibody did not seem to be closely related to the development of tolerance to the toxicity of the antigen.

5. The possible relationship of these findings to certain of the manifestations of typhoid fever are discussed.

The authors are grateful for the advice and constructive criticism of Dr. John F. Enders of the Department of Bacteriology of the Harvard Medical School.

BIBLIOGRAPHY

1. Boivin, A., Mesrobianu, I., and Mesrobianu, L., Technique pour la préparation des polysaccharides microbiens spécifiques. *Compt. rend. Soc. de Biol.*, 1933, 113, 490.
2. Raistrick, H., and Topley, W. W. C., Immunizing fractions isolated from *Bact. aertrycke*. *Brit. J. Exper. Path.*, 1934, 15, 113.
3. Morgan, H. R., Preparation of an antigenic material inducing leucopenia from *Eberthella typhosa* cultured in a synthetic medium. *Proc. Soc. Exper. Biol. and Med.*, 1940, 43, 529.
4. Morgan, H. R., Immunologic properties of an antigenic material isolated from *Eberthella typhosa*. *J. Immunol.*, 1941, 41, 161.
5. Cundiff, R. J., and Morgan, H. R., The inhibition of the bactericidal power of human and animal sera by antigenic substances obtained from organisms of the typhoid-Salmonella group. *J. Immunol.*, 1941, 42, 361.
6. Morgan, H. R. (Unpublished experiments.)
7. Morgan, H. R. (In press.)
8. Kagan, B. M., A simple method for the estimation of total protein content of the plasma and serum. II. The estimation of total protein content of human plasma and serum by the use of the falling drop method. *J. Clin. Invest.*, 1938, 17, 373.
9. Karr, W. G., A method for the determination of blood urea nitrogen. *J. Lab. and Clin. Med.*, 1924, 9, 329.
10. Folin, O., *Laboratory Manual of Biological Chemistry*. D. Appleton-Century Co., Inc., New York, 1934.
11. Folin, O., Two revised copper methods for blood sugar determinations. *J. Biol. Chem.*, 1929, 82, 83.
12. Whitehorn, J. C., Simplified method for the determination of chlorides in blood or plasma. *J. Biol. Chem.*, 1921, 45, 449.
13. Van Slyke, D. D., and Cullen, G. E., Studies of acidosis. I. The bicarbonate concentration of the blood plasma; its significance and its determination as a measure of acidosis. *J. Biol. Chem.*, 1917, 30, 289. II. A method for the determination of carbon dioxide and carbonates in solution. *Ibid.*, 347.
14. Schilling, V., *The Blood and Its Clinical Significance*. C. V. Mosby Co., St. Louis, 1929.
15. Holmes, W. H., *Bacillary and Rickettsial Infections*. The Macmillan Co., New York, 1940.
16. Cowie, D. M., and Calhoun, H., Nonspecific therapy in arthritis and infections. *Arch. Int. Med.*, 1919, 23, 69.
17. Perera, G. A., Clinical and physiologic characteristics of chill. *Arch. Int. Med.*, 1941, 68, 241.
18. Garrey, W. E., and Bryan, W. R., Variations in white blood cell counts. *Physiol. Rev.*, 1935, 15, 597.
19. Pepper, O. H., and Miller, T. G., The relation of allantoin excretion to leukopenia and leucocytosis in rabbits. *J. Infect. Dis.*, 1916, 19, 694.
20. Dennis, E. W., and Senekjian, H., A leucocidal toxin extracted from typhoid bacilli. *Am. J. Hyg. (sect. B)*, 1939, 30, 103.
21. Delafield, M. E., Blood sugar changes and toxic effects produced in rabbits by certain fractions derived from *B. aertrycke*. *Brit. J. Exper. Path.*, 1934, 15, 130.

THE PRODUCTION OF CARDIAC LESIONS BY REPEATED INJECTIONS OF DESOXYCORTICOSTERONE ACETATE^{1, 2, 3}

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Desoxycorticosterone acetate prolongs the lives and restores many of the functions of patients suffering from Addison's disease and of adrenalectomized animals (1 to 5). The beneficial effects are related to the action on the kidneys which leads to excretion of potassium and retention of sodium (1, 5). Clinical observations have suggested that injurious results due to the steroid may appear along with the beneficial effects. While receiving desoxycorticosterone acetate, certain patients develop edema which has been attributed to excessive nephrogenic retention of sodium chloride and water (3, 5). Furthermore, a number of these patients have developed cardiac symptoms, including advanced congestive failure and enlargement of the heart by roentgenogram (2 to 8). In some cases the cardiac decompensation was regarded as a manifestation of previously existing disease of the heart which was aggravated by a return of the blood pressure to normal or high values (3). Certain authors have attributed cardiac failure to the added burden brought about by the increased volume of circulating fluids which retention of sodium chloride by the kidneys has caused (5). McGavack (6) pointed out that continued treatment leads to low concentration of potassium in serum and that this abnormality may account for the heart failure. Since it is recognized that desoxycorticosterone acetate exerts little or none of the glycogenic function of the adrenal cortex, disturbances in carbohydrate metabolism might be anticipated in Addisonian patients treated with the synthetic hormone (9). Hypoglycemia has been observed but does not seem to be an important factor except during pro-

longed fasting (9). Cardiac failure remains unexplained and seems to be the chief untoward effect that accompanies the therapeutic use of desoxycorticosterone acetate in Addison's disease.

In experimental animals, repeated injections of the synthetic compound lead to gross deficits of potassium and abnormal retentions of sodium in skeletal muscle (10, 11). These changes are probably a reflection of a tendency to develop low concentrations of potassium in serum (10, 11, 6). Increased urinary volume which is apparently brought about by increased thirst and drinking is a part of the picture (11 to 13). With prolonged treatment with large doses in dogs, a peculiar muscular paralysis develops which is relieved by administration of potassium salts (11). The injection of desoxycorticosterone acetate has not been shown to produce cardiac symptoms in experimental animals (14).

On the other hand, diets low in potassium produce the same changes in skeletal muscle as have been demonstrated after repeated injections of desoxycorticosterone acetate (15, 16) and cardiac lesions are strikingly developed in rats (16 to 19), mice (20), and pigs (19), fed diets deficient in potassium. Certain observations (19) lead to the conclusion that a dietary deficiency in pyridoxin played a rôle in the development of the cardiac lesions accompanying diets low in potassium. The present paper reports work which demonstrates that cardiac lesions are produced by prolonged injections of desoxycorticosterone acetate in rats. The lesions are not prevented by a relative excess of thiamin or pyridoxin in synthetic diets, nor are the lesions aggravated by sub-optimal amounts of these vitamins in the diets. Analyses of muscle and serum are reported to show the effect of desoxycorticosterone acetate in rats. In cats and dogs, analyses of other tissues are given, with especial attention to the variations of potassium in the heart.

¹ Technical assistance made possible by a grant from Mead Johnson and Company.

² Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

³ We are indebted to Ciba Products, Inc., for the desoxycorticosterone acetate and to the Upjohn Company for the adrenocortical extract used in the experiments.

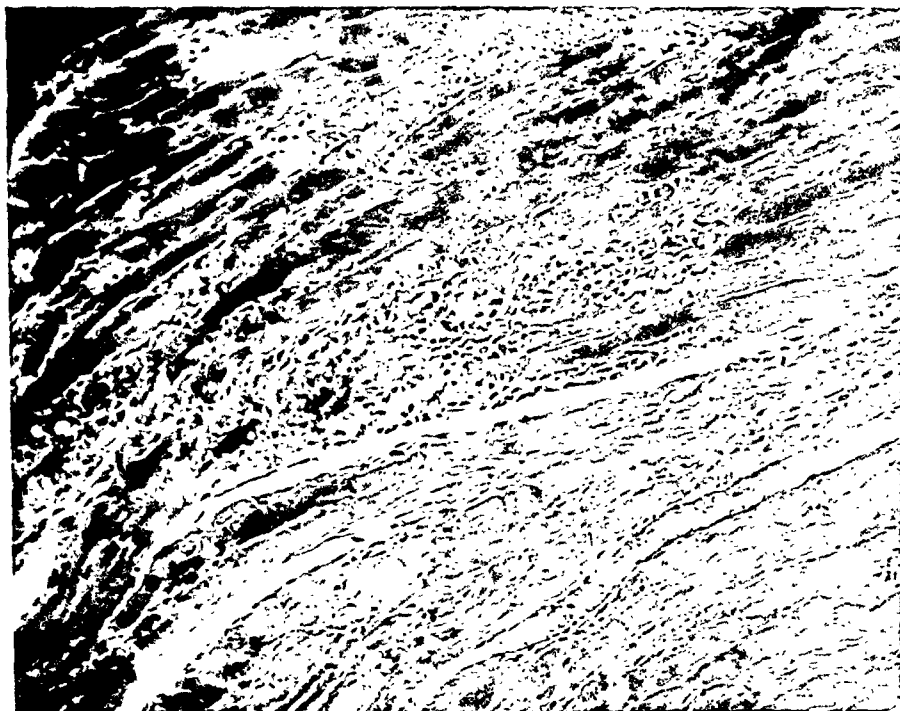


FIG. 2. CARDIAC LESIONS PRODUCED BY INJECTIONS OF DESOXYCORTICOSTERONE ACETATE
Rat. 155. Fed Purina Fox Chow. Injected with 4 mgm. of desoxycorticosterone for
30 days.



FIG. 3. CARDIAC LESIONS PRODUCED BY INJECTIONS OF DESOXYCORTICOSTERONE ACETATE
Cat 2. Received 8 mg. desoxycorticosterone acetate daily for 25 days.

METHODS

White male rats weighing 200 to 350 grams were used. The desoxycorticosterone acetate was dissolved in warm alcohol and then precipitated by adding enough 5 per cent glucose or saline to make a suspension in 5 per cent alcohol containing 2 mgm. of the synthetic hormone per ml. This mixture was injected subcutaneously in 1, 2, or 4 mgm. doses daily except Sundays. The rats were killed by withdrawing as much blood as possible from the abdominal aorta while the rat was under ether anesthesia. Muscle or other tissue were removed immediately and analyzed as in previous studies (21). Serum analyses were obtained from blood kept under mineral oil until separated from the erythrocytes.

The stock rats were fed Purina Fox Chow and this diet was continued during many of the experiments. By analysis this food contains 15 millimoles of potassium per 100 grams. Two basic synthetic diets were used for rats. Diet A contained the following: vitamin free casein (Labco) 180 grams, sucrose 630, Crisco 100, cod liver oil 10, liver B fraction 20, soya bean oil 20, salt mixture 40. To a kilogram of the diet the following vitamins were added: thiamin 20 mgm., riboflavin 20 mgm., nicotinic acid 100 mgm., calcium pantothenate 20 mgm., and cholin 1 gram. Three variations of the above were made: (1) no added pyridoxin (OB6), (2) 2 mgm. pyridoxin (NB6), and (3) 20 mgm. pyridoxin (HB6). The salt mixture gave 15 millimoles of potassium per 100 grains of diet.

Diet A was intended to give liberal amounts of the vitamins and study the effect of variations in pyridoxin. None of the rats, even on the diet free of B6, showed acrodynia. This was probably due to the presence of soya bean oil (22). Diet B contained the following: Labco casein 180 grams, sucrose 630, Crisco 100, cod liver oil 10, liver B fraction 20, soya bean oil 20, salt mixture 40. The following vitamins were added to 1 kgm.: riboflavin 20 mgm., nicotinic acid 100 mgm., calcium pantothenate 20 mgm., pyridoxin 20 mgm., cholin 1 gram. To one lot, 2 mgm. of thiamin were added (NB1), and to another, 0.5 mgm. of thiamin (LB1). Diet B was intended to study the effects of low intakes of thiamin. The potassium content of this diet was 15 millimoles per 100 grams.

The cats were fed canned salmon and milk. From analyses of the salmon, it was calculated that the intake of potassium was rather high,—about 11 millimoles per kilogram of cat. The dogs were given a synthetic diet low in potassium made up of casein, sugar, fats, vitamins, and a salt mixture free of potassium. The dog injected with desoxycorticosterone acetate received the regular kennel diet.

RESULTS

The photomicrographs illustrate the cardiac lesions produced by injections of desoxycorticosterone acetate (Figures 1, 2, 3). For comparison, lesions produced by a diet low in potassium

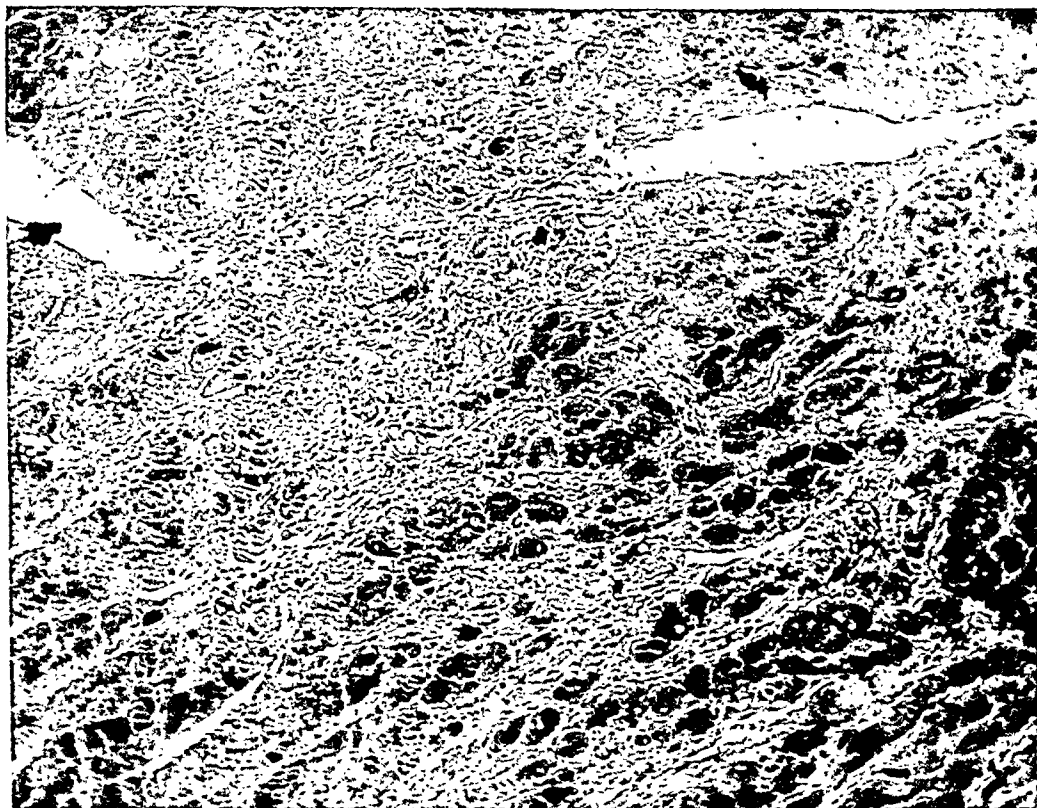


FIG. 1. CARDIAC LESIONS PRODUCED BY INJECTIONS OF DESOXYCORTICOSTERONE ACETATE
Rat. 151. Fed Purina Fox Chow. Injected with 4 mgm. of desoxycorticosterone acetate for 30 days. Diet contains 24 mM. sodium and 15 mM. potassium per 100 grams.

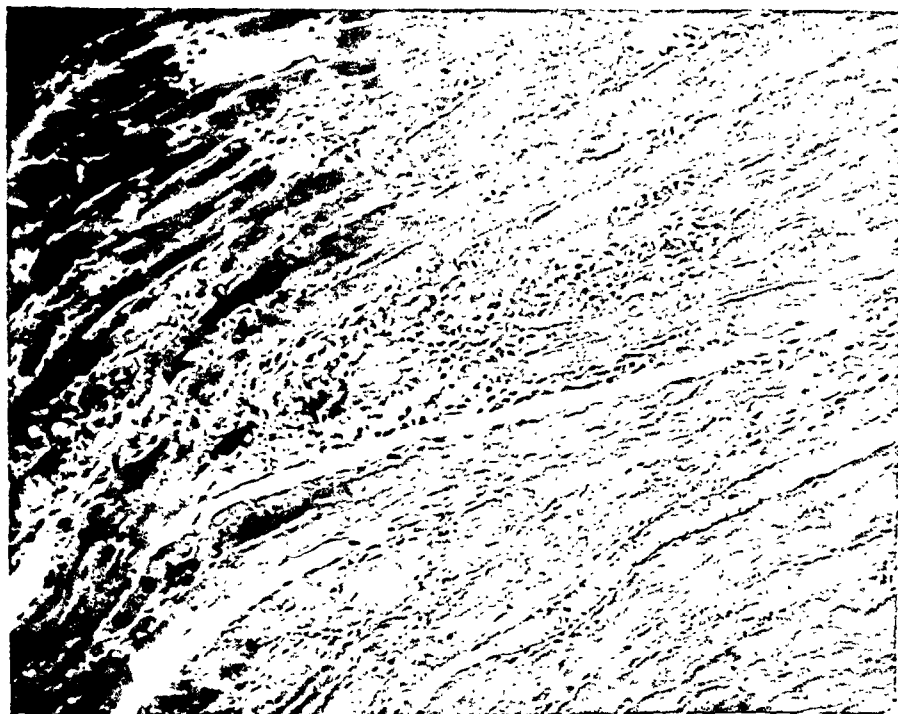


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FIG. 4. CARDIAC LESIONS PRODUCED BY A DIET LOW IN POTASSIUM

Rat M 42. Fed diet low in potassium for 69 days. Diet contains 1.6 mM. potassium and 18 mM. sodium per 100 grams.

are also reproduced (Figure 4). Essentially, there are small or large areas of necrosis of cardiac muscle fibres which are replaced by fibrous connective tissue. There is no evidence of infiltration by polymorphonuclear leukocytes. Skeletal muscle, diaphragm, and liver have been examined but do not show lesions. The kidney weights are increased (23) and the tubules enlarged, with multiplication of tubular cells. Both the heart and renal lesions cannot be distinguished from the lesions seen by us and others in rats fed a diet low in potassium (16 to 20).

Cats 2 and 4 were examined histologically. Cat 2 showed typical lesions in the heart which are regarded as produced by the desoxycorticosterone, in spite of the fact that this cat was suffering from an infection and was very sick and refused to eat during the last 4 days of her life. Dogs 586 and 802 were examined histologically and showed no lesions in the heart. The kidneys, however, of both the cats and dogs show lesions in the tubules like those seen in the rats, but much less marked.

Table I shows the incidence of lesions in rats

fed the various diets and injected with various doses of desoxycorticosterone acetate. One unmistakable area of replacement of musculature by fibroblasts was considered sufficient to indicate

TABLE I
Incidence of cardiac lesions

Diet		DOCA		Number of rats	Number with lesions
Kind ¹	Vitamin ²	mgm.	days		
P		2	14	7	1
P		2	30	12	9
P		4	30	10	9
A	OB6	1	30	4	2
A	OB6	2	30	2	0
A	OB6	4	30	2	2
A	NB6	2	30	4	2
A	NB6	4	30	4	2
A	HB6	2	30	1	1
A	HB6	4	30	3	3
B	LB1	4	30	4	2
B	NB1	4	30	4	2

¹ P indicates Purina Fox Chow; A and B, the synthetic diets described under methods.

² The following vitamin supplements were added to a kilogram of diet: OB6, no pyridoxin; NB6, 2 mgm. pyridoxin; HB6, 20 mgm. pyridoxin; LB1, 0.5 mgm. thiamin; HB1, 2 mgm. thiamin. See text for composition of A and B.

that lesions were produced. In most instances, only one section was examined but experience with two separate imbeddings indicates that striking lesions might be found in one section when absent in the other. Hence positive findings are more significant than negative findings. Although some hearts are obviously more injured than others, we were unable to satisfy ourselves that we were justified in laying emphasis on quantitative interpretation. The data will, therefore, be considered merely on the basis of lesions found or not found.

Of sections examined from 7 rats given 2 mgm. of desoxycorticosterone acetate daily for 14 days, one showed lesions. In most experiments, the injections were continued for 30 or 40 days. Since no obvious increase in the incidence of lesions developed between the thirtieth and fortieth day and most of the experiments were run for 30 days, all the longer experiments are listed as injected for 30 days. For the prolonged period of injections, lesions were found in 34 of 50 rats. Lesions were present when the daily dose was 1, 2, or 4 mgm. There was a suggestion that the lesions were more marked and more frequent in the rats given the larger doses.

Because it has been suggested that the cardiac lesions produced by diets low in potassium are ag-

gravated by a deficiency in pyridoxin, experiments were set up to test this possibility for the injury produced by injections of desoxycorticosterone acetate (A diets). The diets contained rather liberal amounts of added thiamin, riboflavin, nicotinic acid, calcium pantothenate, cholin and liver B fraction. Diets were tested containing no pyridoxin, an adequate, and a high level. Pyridoxin intake did not apparently affect the production of lesions.

In the experiments of Thomas *et al.* (19) smaller doses of thiamin were given than the amount obtained from our A diets. These authors found evidence that the cardiac lesions produced by diets low in potassium were aggravated by a deficiency in pyridoxin. The B diets were, therefore, made up to test whether minimal and suboptimal amounts of thiamin brought out more extensive lesions than those previously obtained on the A diets. As may be seen from the table the level of intake of thiamin did not influence the cardiac lesions produced by prolonged injections of desoxycorticosterone acetate.

Purina Fox Chow was supplemented in three ways by adding (1) 20 grams of soya bean oil, (2) 20 mgm. of pyridoxin, and (3) 20 mgm. of thiamin per kilogram of ground material. These results are not included in the tables. In each

TABLE II
Serum and muscle of rats

	Number of rats	Serum				Muscle per 100 grams fat free solids					
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Normal	13	grams 92.5 ±0.6	mM. 4.0 ±0.8	mM. 113.0 ±3.3	mM. 146.9 ±3.0	grams 341 ±6.6	mM. 7.2 0.5	mM. 10.0 0.6	mM. 48.9 ±0.6	mM. 33.4 ±1.5	grams 14.8 ±0.3
DOCA 10	21	94.1 ±0.6	3.39 ±0.8	109.9 ±3.1	150.0 ±6.2	332 ±9.0	6.27 ±0.7	14.4 ±1.5	41.6 ±2.4	32.0 ±1.1	15.4 ±0.1
DOCA 30	21	94.1 ±0.8	4.23 ±1.1	104.7 ±4.9	149.7 ±4.7	332 ±8.7	6.48 ±0.7	16.6 ±2.0	38.4 ±2.5	32.1 ±0.9	15.3 ±0.3
LK 14	15	93.2 ±1.2	4.5 ±0.8	113.4 ±2.5	146.3 ±5.4	327 ±5.5	6.3 ±0.7	11.8 ±0.8	40.3 ±2.1	31.3 ±0.7	15.2 ±0.2
C.E. 31	1	93.9		111	149	329	6.0	10.9	43.0		15.3

In this and subsequent tables, average \pm standard deviations are given.

DOCA 10—rats getting 2 mgm. desoxycorticosterone for 10 to 14 days.

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Rat M 42. Fed diet low in potassium for 69 days. Diet contains 1.6 mM. potassium and 18 mM. sodium per 100 grams.

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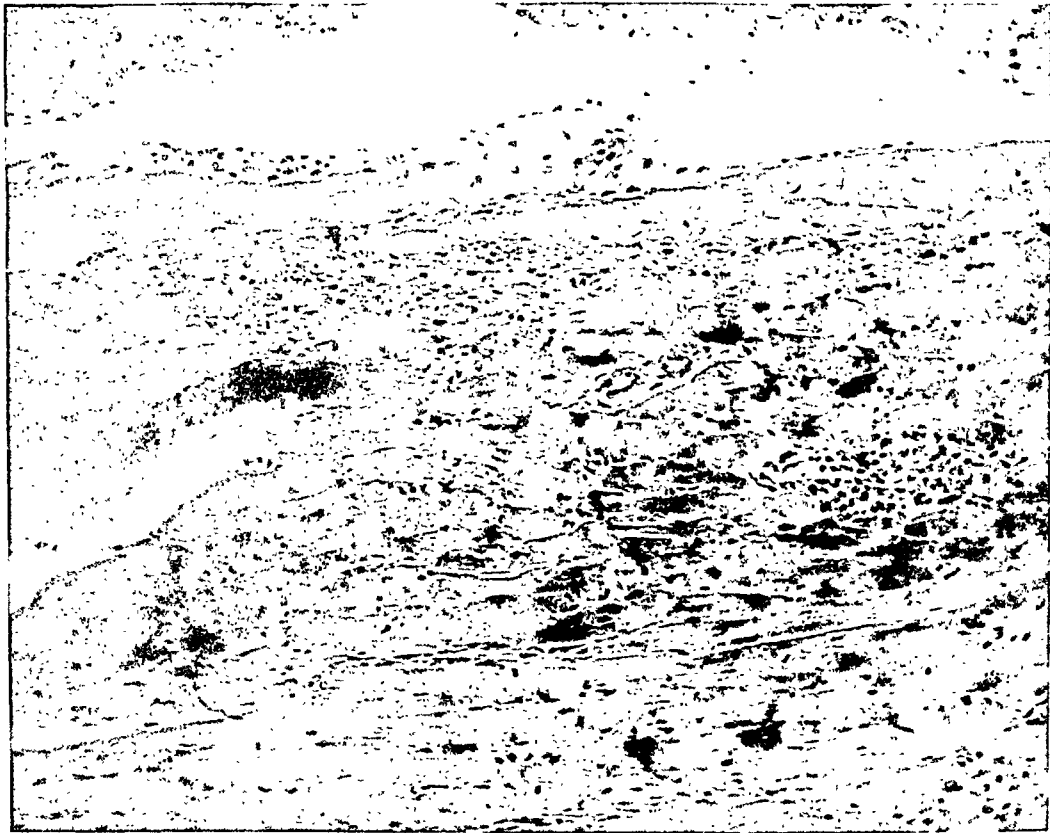


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TABLE VI
Serum, muscle, and heart in cats receiving desoxycorticosterone

	DOCA	Days	Serum				Tissue					
			per 100 ml.	per L.	per L. ultrafiltrate		per 100 grams fat free solids					
			H ₂ O	K	Cl	Na	H ₂ O	Cl	Na	K	P	N
	mgm.		grams	mM.	mM.	mM.	grams	mM.	mM.	mM.	mM.	grams
MUSCLE												
Controls			93.2 ±0.6	5.7 ±0.3	132.6 ±3.4	155.8 ±3.4	345 ±18	5.9 ±1.1	8.0 ±1.1	47.4 ±2.1	33.5 ±1.5	15.4 0.2
Cat 2	8	25	93.5	4.4	124		339	14	16	34	35	16.2
Cat 3	4	11	93.3	5.3	132	157	329	5.8	9.0	45	31	14.7
Cat 4	4	35	91.5	4.0	131	163	320	7.8	11.3	48	31	15.4
Cat 5	4	11	93.9	4.8	136	154	327	5.6	8.1	46	32	15.3
HEART												
Controls							410 ±27	19.1 ±1.9	24.4 ±4.4	40.1 ±3.4	34.0 ±2.9	14.1 ±0.9
Cat 2	8	25					429	27	36	48	35	16.0
Cat 3	4	11					395	16	22	39	35	14.0
Cat 4	4	35					381	25	28	40	32	14.8
Cat 5	4	11					426	21	23	44	37	14.9

quate amounts of the known vitamins and 15 mM. of potassium per 100 grams. The low potassium diet was controlled by adding 0.5 per cent potassium chloride to the drinking water of a group on the diet low in potassium. The table shows that the cardiac potassium is slightly lower in the rats receiving desoxycorticosterone acetate than in the control group, and lower in the ones receiving a diet low in potassium than in the rats receiving the same diet plus 0.5 per cent potassium chloride in drinking water. The rats receiving desoxycorticosterone acetate also have slightly high cardiac sodium. The figures suggest that heart muscle reacts somewhat like skeletal muscle, but the changes are certainly not as large proportionately as those found in skeletal muscle.

Because of difficulty in analysing small amounts of tissue, procedures that reduce body potassium were studied in dogs and cats. Table V shows the results in heart and muscle of 4 dogs receiving a diet low in potassium, and 1 receiving daily injections of 10 mgm. of desoxycorticosterone acetate for 14 days. Analogous changes to those found in rat muscle also develop in dog muscle. In 2 of the 4 hearts of dogs receiving the diet low in potassium, distinctly low potassium developed (see dogs 586 and 651). The decrease equals one-fourth of the usual cardiac potassium. These are also the dogs showing the lowest potassium in skeletal muscle. The other 2 hearts of the dogs

on the diet low in potassium were essentially normal. Following injections of desoxycorticosterone acetate for 14 days, the cardiac potassium was slightly low but not certainly below the normal range. The results show that procedures leading to loss of body potassium may lead to considerable reduction of cardiac potassium in the dog. However, the same procedure does not lead to this change in all animals.

Table VI shows the results following daily injections of desoxycorticosterone in cats. None of the hearts show significant loss of cardiac potassium. Cat 2 which was very sick, in part owing to an infection, shows high water, chloride, and sodium in the heart. This cat also is the only one showing the characteristic loss of potassium in skeletal muscle. The positive result may be due to the larger dose in this cat. However, the relative refractoriness of the cat to desoxycorticosterone may be more apparent than real. Cat 4 apparently shows the effect of desoxycorticosterone acetate in raising the concentration of sodium in serum. The diet of the cats consisted largely of rather salty canned salmon and by analysis of the diet it was calculated that a 2 kilogram cat got about 11 mM. of potassium and 20 mM. of sodium per day. Per 100 grams, the cat's diet contained 30 and 54 mM. of potassium and sodium respectively, which is about twice as concentrated in potassium as the rats' diets.

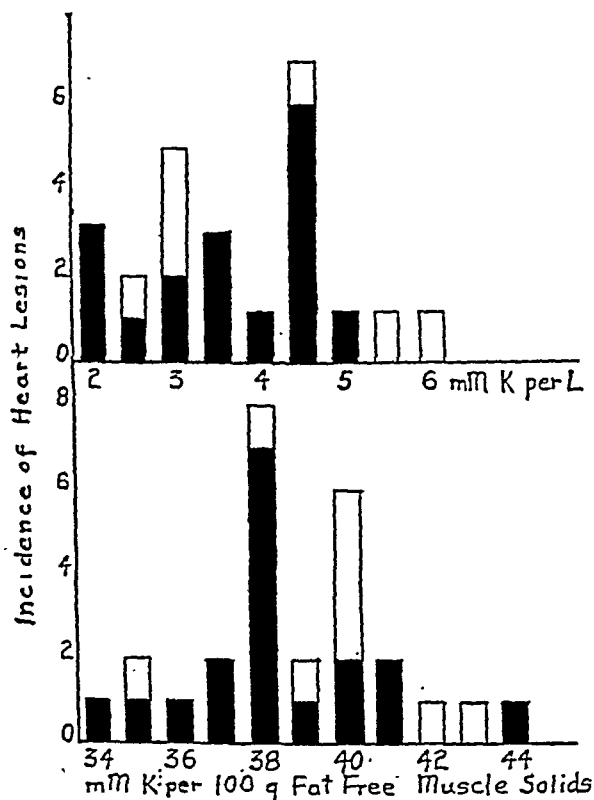


FIG. 5. THE INCIDENCE OF CARDIAC LESIONS WITH RESPECT TO POTASSIUM CONCENTRATION IN SERUM AND IN MUSCLE

The height of the columns indicates the number of rats examined and the black areas the number with cardiac lesions. In the upper part the rats are classified with respect to serum potassium, while in the lower part they are classified with respect to muscle potassium.

Since cats are so particular about their diet, they are probably not suitable for demonstrating deficits of potassium. However, the experiments support the evidence that deficits of potassium are necessary for the production of lesions in the heart.

In Figure 5, the incidence of cardiac lesions is charted against the concentration of potassium per 100 grams of fat free solids of skeletal muscle, and against the concentration of potassium in serum. The chart includes all data on rats obtained from experiments involving diets low in potassium and the injection of desoxycorticosterone acetate, in which serum and muscle analyses were made in conjunction with histological examination of the heart. It should be recalled that the authors have pointed out that muscle

potassium in normal rats may vary from 44 to 49 mM. per 100 grams of fat-free solids. When muscle potassium is above 50 mM., serum potassium is high, and when it is below 44 mM., serum potassium has a tendency to be low. Since the concentration of potassium in serum is subject to many sudden and transient variations, it is not surprising that the values in the chart are usually in the normal range but with a tendency for a considerable number to be abnormally low. There is no consistent correlation between the level of serum potassium and the incidence of cardiac lesions. On the other hand, all of the muscle potassiums but one lie below the normal range. The lesions in the heart were found at all levels of muscle potassium from 34 to 44 mM. per 100 grams of fat free solids. Lesions occurred, with few exceptions, when muscle potassium was below 40. Thus the degree of depletion of body potassium, as indicated by the diminution of muscle potassium, is an index of the incidence of cardiac lesions.

DISCUSSION

The lesions in the hearts of the rats are of sufficient severity to explain cardiac failure. Although circulatory insufficiency and cardiac dilatation are the chief untoward results of inadequately controlled treatment with desoxycorticosterone acetate, reports of autopsies in patients so treated have not described lesions like those under consideration. In the first clinical trials when the most cardiac symptoms were encountered, administration of desoxycorticosterone acetate was often combined with diets low in potassium and high in sodium chloride. Undoubtedly this practice aggravates the tendency to lose potassium and the consequent untoward cardiac symptoms. In a few rats, sodium chloride was added to the drinking water. While extensive lesions were produced in this way, the rats run at the same time but receiving tap water showed apparently equally severe lesions. As was pointed out previously, the diet of the cats was high in both potassium and sodium, containing 30 and 50 mM. respectively per 100 grams of diet. These figures may be compared to the rat diet which contained per 100 grams, 15 and 8.5 mM. of potassium and sodium. This peculiarity of the cat diet probably

explains the normal concentration of potassium in serum and muscle as well as the high concentration of sodium in serum. That the dietary factor may be decisive is suggested by the fact that cat 2, which ate poorly, developed deficit of potassium in muscle. Furthermore, addition of 0.5 per cent potassium chloride to the drinking water of rats receiving desoxycorticosterone acetate prevented renal hypertrophy, hyperplasia of renal tubules, loss of potassium from the muscle, and cardiac lesions. The use of desoxycorticosterone acetate in Addison's disease must be controlled so that deficits of potassium are not produced.

In evaluating the present experiments, it must be kept in mind that all animals had intact adrenals. The dose of desoxycorticosterone acetate was 1, 2, and 4 mgm. daily, which is only 2 to 8 times the dose for maintenance of an adrenalectomized rat. If patients are as susceptible to cardiac injury as the rat, therapeutic doses are probably not far below the amount that might be injurious. Probably the therapeutic dose will be found to depend in some way on the intake of both potassium and sodium and by striking a correct balance, the margin of safety can be increased. The diet of the rats contained about 4.4 mM. per 100 calories; a human dietary of 2000 calories and 4 grams of potassium contains about 5.0 mM. of potassium per 100 calories. With loss of appetite and the use of intravenous therapy, potassium intake may seriously decrease and administration of desoxycorticosterone acetate lead to deficits of potassium. In any case, the synthetic hormone should not be used with diets low in potassium or with those containing excessive amounts of sodium chloride.

While only one rat was analysed after receiving adrenal cortical extract, the muscles in this case showed low potassium. Loss of body potassium cannot, therefore, be regarded as a peculiar effect of a synthetic compound. Indeed, one of us (H. C. M.) has unpublished data which show that injections of estradiol benzoate and testosterone propionate produce low muscle potassium. Furthermore, the effects of diets low in potassium show that the kidneys are unable entirely to reabsorb potassium from the glomerular filtrate. Renal hypertrophy enables the tubules to be more successful (18), and the renal hypertrophy of rats

receiving desoxycorticosterone acetate is probably entirely analogous to that of rats receiving diets low in potassium (23), despite the fact that the ones receiving the hormone have high urinary volumes and those on the diets low in potassium have low urinary volumes. Apparently under the influence of certain steroids, the tendency not to reabsorb potassium from the glomerular filtrate is so great as to lead to body deficits, even on diets adequate in potassium.

At present no simple laboratory method will surely indicate when a deficit of potassium has developed. In the cats, the abnormally high concentration of sodium in serum was accompanied by normal potassium in muscle and serum; serious deficits of potassium may occur with normal concentrations of this ion in serum in rats. Probably concentration of potassium in serum below 3.5 mM. per liter should be regarded as dangerous. Therapy should not be pushed with only the concentration of sodium or chloride in serum as the criterion of adequate administration; nor does a normal concentration of potassium in serum give assurance that deficit of body potassium has not developed.

Apparently treatment of Addison's disease has met a dilemma. During a crisis, the Addisonian patient is extremely susceptible to the toxic effects of potassium, yet with treatment with desoxycorticosterone acetate he may develop a harmful deficit of potassium. Although renal excretion of sodium and potassium may be related to one another in a reciprocal way, the balance in the body depends on the intake of each independently. Without the hormone, the Addisonian patient tends to lose sodium and retain potassium; a patient receiving desoxycorticosterone acetate tends to lose potassium and retain sodium. Doubtless future work can show what balance between intake of sodium and potassium is the most favorable in the treatment of adrenal insufficiency with desoxycorticosterone acetate.

SUMMARY

Necrosis of the myocardial fibres and replacement by fibroblasts is produced by repeated injections of desoxycorticosterone acetate in rats. The lesions are neither aggravated by absence of pyridoxin nor prevented by liberal additions of

pyridoxin to the diets. Low intake of thiamin does not aggravate the lesion. The lesions cannot be distinguished from those produced by diets low in potassium. The livers decrease in size after injections of desoxycorticosterone acetate for 10 days, but are normal in size after 4 weeks of injections.

The injection of desoxycorticosterone acetate lowers muscle potassium and raises muscle sodium. Analogous changes are not found in the liver. Low cardiac potassium was found in the heart in 2 of 4 dogs fed a diet low in potassium. Injection of desoxycorticosterone produced only suggestive lowering of cardiac potassium in a group of rats, no certain change in any of 4 cats, and no change in 1 dog. Although the heart may lose potassium under conditions leading to loss from skeletal muscle, diminution of cardiac potassium is not a regular occurrence.

The cardiac lesions produced by injections of desoxycorticosterone acetate or diets low in potassium can be prevented by addition of potassium chloride to the drinking water. Deficit of body potassium is apparently essential for the production of these lesions.

Cortical extract produced analogous changes in the muscle of 1 rat.

REFERENCES

1. Thorn, G. W., Howard, R. P., and Emerson, K., Jr., Treatment of Addison's disease with desoxycorticosterone acetate, a synthetic adrenal cortical hormone. *J. Clin. Invest.*, 1939, 18, 449.
2. Thorn, G. W., and Firor, W. M., Desoxycorticosterone acetate therapy in Addison's disease. *J. A. M. A.*, 1940, 114, 2517.
3. Thorn, G. W., The treatment of Addison's disease. *J. Clin. Endocrinol.*, 1941, 1, 76.
4. McCullagh, E. P., and Ryan, E. J., The use of desoxycorticosterone acetate in Addison's disease. *J. A. M. A.*, 1940, 114, 2530.
5. Ferrebee, J. W., Ragan, C., Atchley, D. W., and Loeb, R., Desoxycorticosterone esters; certain effects in the treatment of Addison's disease. *J. A. M. A.*, 1939, 113, 1725.
6. McGavack, T. H., Some pitfalls in the treatment of Addison's disease. *J. Clin. Endocrinol.*, 1941, 1, 68.
7. Gordon, E. S., The use of desoxycorticosterone and its esters in the treatment of Addison's disease. *J. A. M. A.*, 1940, 114, 2549.
8. Willson, D. M., Ryncarson, E. H., and Dry, T. J., Cardiac failure following treatment of Addison's disease with desoxycorticosterone acetate. *Proc. Staff Meet., Mayo Clin.*, 1941, 16, 168.
9. Thorn, G. W., Koepf, G. F., Lewis, R. A., and Olsen, E. F., Carbohydrate metabolism in Addison's disease. *J. Clin. Invest.*, 1940, 19, 813.
10. Miller, H. C., and Darrow, D. C., Relation of serum and muscle electrolyte, particularly potassium, to voluntary exercise. *Am. J. Physiol.*, 1941, 132, 801.
11. Ferrebee, J. W., Parker, D., Carnes, W. H., Gerity, M. K., Atchely, D. W., and Loeb, R. F., Certain effects of desoxycorticosterone: The development of "diabetes insipidus" and the replacement of muscle potassium by sodium in normal dogs. *Am. J. Physiol.*, 1941, 135, 230.
12. Ragan, C., Ferrebee, J. W., Phyfe, P., Atchely, D. W., and Loeb, R., A syndrome of polydypsia and polyuria induced in normal animals by desoxycorticosterone acetate. *Am. J. Physiol.*, 1940, 131, 73.
13. Mulinos, M. G., Spingarn, C. L., and Lojkin, M. E., A diabetes insipidus-like condition produced by small doses of desoxycorticosterone acetate in dogs. *Am. J. Physiol.*, 1941, 135, 102.
14. Carnes, W. H., Ragan, C., Ferrebee, J. W., and O'Neil, J., Effects of desoxycorticosterone acetate in albino rat. *Endocrinol.*, 1941, 29, 144.
15. Heppel, L. A., Electrolytes of muscle and liver in potassium-depleted rats. *Am. J. Physiol.*, 1939, 127, 385.
16. Miller, H. C., and Darrow, D. C., Relation of muscle electrolyte to alterations in serum potassium and to the toxic effects of injected potassium chloride. *Am. J. Physiol.*, 1940, 130, 747.
17. Schrader, G. A., Prickett, C. O., and Salmon, W. D., Symptomatology and pathology of potassium and magnesium deficiencies in rats. *J. Nutrition*, 1937, 14, 85.
18. Follis, R. H., Jr., Orent-Keiles, E., and McCollum, E. V., The production of cardiac and renal lesions in rats by a diet extremely deficient in potassium. *Am. J. Path.*, 1942, 18, 29.
19. Thomas, R. M., Mylon, E., and Winternitz, M. C., Myocardial lesions resulting from dietary deficiency. *Yale J. Biol. and Med.*, 1940, 12, 345.
20. Liebow, A. A., McFarland, W. J., and Tennant, R., The effects of potassium deficiency on tumor-bearing mice. *Yale J. Biol. and Med.*, 1941, 13, 523.
21. Darrow, D. C., Harrison, H. E., and Taffel, M., Tissue electrolytes in adrenal insufficiency. *J. Biol. Chem.*, 1939, 130, 487.
22. Schneider, H. A., Ascham, J. K., Platz, B. R., and Steenbock, H., The antiacrodynic properties of certain foods. *J. Nutrition*, 1939, 18, 99.
23. Durlacher, S. H., Darrow, D. C., and Winternitz, M. C., The effect of low potassium diet and of desoxycorticosterone acetate upon renal size. *Am. J. Physiol.*, 1942, 136, 346.

THE VELOCITY OF BLOOD FLOW IN INFANTS AND YOUNG CHILDREN, DETERMINED BY RADIOACTIVE SODIUM¹

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To measure the velocity of blood flow in infants and young children, a completely objective procedure is required. Most of the methods (as for example determining the time elapsed between the injection of a sweet substance (saccharin) or a bitter substance (sodium dehydrocholate) and the arrival on the tongue of its characteristic taste) used for adults need the cooperation of the subject.

The respiratory stimulation caused by potassium cyanide, injected intravenously, was utilized as an objective method by Robb and Weiss (1). We have not tried to apply this method to infants because of the danger in adjusting small doses to small patients. The lethal dose is said to be only about ten times the stimulating dose. Furthermore, the end point would be difficult to evaluate since the infant's respirations would almost certainly be irregular and disturbed by crying and emotional excitement.

A satisfactory method for infants calls for a test material which can be accurately and objectively measured in small amounts and which, in the doses used, is completely harmless. Radioactive sodium appeared to satisfy these requirements.

The principle of using a radioactive substance was developed by Blumgart and Weiss (2), who injected the active deposit of radon into the antecubital vein of one arm, detecting its arrival in the other arm by a properly shielded cloud chamber. The active deposit consists of radium B and its products in equilibrium. It decays with a half-life of 26 minutes. The dose which they used, usually about 5 millicuries, was so small as to present no immediate danger. However, the residue is radium D, a radioactive isotope of lead, with the very long half-life of 22 years. Although the activity of radium D, resulting from the disintegration of 5 millicuries of active deposit is very

small, about 0.025 microcuries, the fact that lead isotopes are concentrated in bones and are eliminated with difficulty makes doubtful the wisdom of injecting into infants a substance which might over a period of years have a deleterious effect on bone growth.

Radioactive sodium on the other hand has a half-life of 15 hours. After disintegration it leaves a normal, inactive atom and there can be no possible ill effects years later. The disadvantages of radioactive sodium as compared with the active deposit of radon are (1) it must be produced by cyclotron bombardment and is therefore not generally available, (2) its longer half-life means that the initial dose in microcurie hours is larger, (3) circulation rate measurements can be repeated in the same individual only after a couple of days have passed, as opposed to 2 hours with active deposit, and (4) its gamma rays are so penetrating as to present some problems in adequate shielding of the detector. With these limitations, however, the method is feasible, and has given results which appear reliable.

METHODS

The test material was prepared in the Harvard cyclotron by bombarding a layer of c.p. sodium metal 1 mm. thick for one hour with 11.5 million electron volt deuterons (charged nuclei of "heavy" hydrogen) with a beam strength of 10 microamperes. About 1 gram of the activated sodium metal was dissolved in a paraffin-lined beaker in 100 cc. of distilled water under CO₂ gas. After filtering, it was made slightly acid with 5 N HCl, boiled to expel CO₂, and to precipitate any silicates, again filtered, and finally neutralized by addition of dilute NaOH or HCl with bromthymol-blue indicator. It was made up to a concentration of 2 per cent to 5 per cent sodium chloride. The test dose was then contained in less than 1 cc., usually 0.1 to 0.5 cc. Such small amounts of hypertonic solution could not be expected to have any effect on the velocity of blood flow.

The radioactivity of the resulting solution was measured by comparison with the gamma-ray activity of a

¹ Read before the Society for Pediatric Research at Atlantic City, New Jersey, May 7, 1941.

known radium sample using a Geiger counter. It was further checked by determining the counting rate with a counter subtending a known solid angle and using a calculated gamma-ray efficiency factor.

The test dose was calculated on the basis of the subject's weight and varied between 2 and 5 microcuries per kilogram of body weight. Since the radiosodium is distributed rapidly throughout the body, there is no danger of large localized irradiation. The total dose was well within a conservative limit of safety when compared to estimated x-ray tolerance. In order to establish the innocuousness of this dose, preliminary tests were done on volunteer adults. Total and differential blood counts were taken before and at intervals following the injection. The blood counts, which may be considered the most sensitive indication of measurable effect from irradiation (3) showed no significant changes. Much larger doses of radioactive sodium have been given therapeutically to adults with leukemia. Hamilton and Stone (4) have given up to 54 millicuries without resulting blood changes, whereas the dose used here for an adult weighing 80 kilograms would be, at 4 microcuries per kilogram, 320 microcuries or 0.32 millicuries. All of the infants and most of the older children who were tested were hospitalized patients, so that it was possible to observe them closely following the procedure. No effects referable to the procedure were noted in the clinical condition, the blood counts, or urine analyses.

In performing the test, the radioactive sodium chloride solution was first sterilized by boiling and then injected

into one arm while the opposite hand was held close to a Geiger counter, shielded by several inches of lead. From the Geiger counter a Nehr-Harper extinguishing circuit with one stage of voltage amplification, power amplifier, and pulse-lengthening circuit was arranged so as to record the arrival of the radioactive material on a waxed tape running at constant speed.

The Geiger counter has unfortunately a certain "background" counting rate, due to cosmic rays and radioactive materials in the walls of the building, etc. In each case before the injection was made, the background rate which averaged about 1 count each 2 seconds was measured over a period of 1 minute. The intravenous injection was then made by an assistant and the time of the injection marked on the recording tape by means of a circuit from a telegraph key. The tape was allowed to run at its constant rate until a few moments after the arrival in the hand of the radioactive material, which was signaled by the rapid increase in the counting rate.

From the record on the tape, spot graphs were made (Figure 1), each recorded count being represented by a dot in the proper position along the time axis. Each successive dot was put one coordinate unit above the preceding dot so that the ordinate equaled the total number of counts up to any given time. The slope of a straight line drawn through these dots represents the counting rate itself. The arrival of the radioactive material is thus indicated by a sharp rise in the slope. The time interval in seconds between the injection and this rise in slope then denotes the circulation rate.

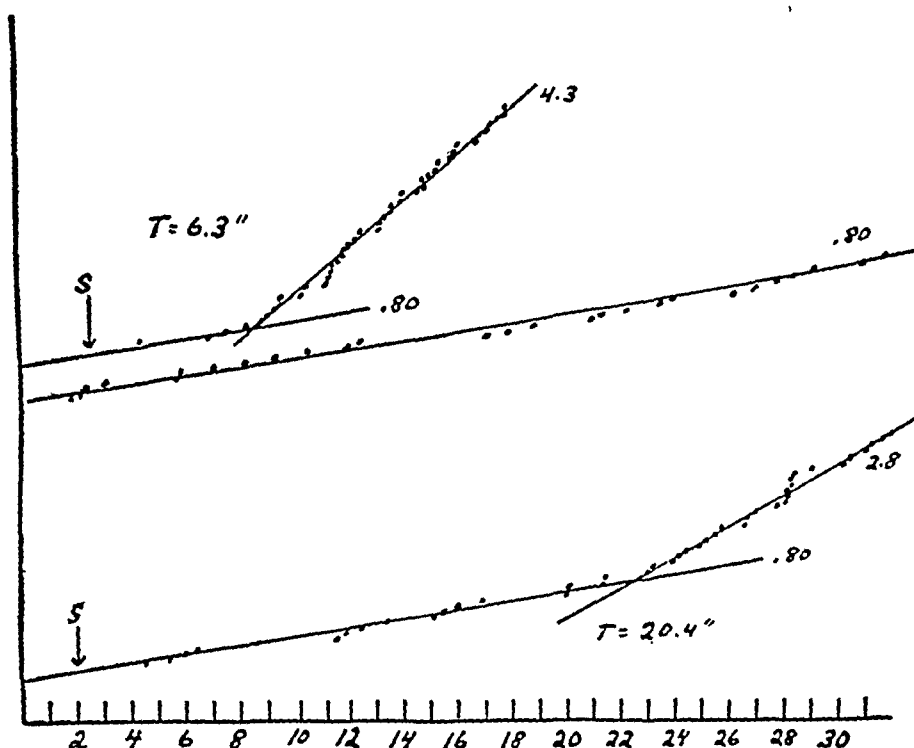


FIG. 1. SPOT GRAPH OF 2 TESTS SHOWING CIRCULATION RATE AS THE TIME IN SECONDS BETWEEN THE INJECTION (S) AND THE SUDDEN INCREASE IN THE FREQUENCY OF THE COUNTS DENOTED AS AN ABRUPT RISE IN SLOPE OF A LINE DRAWN THROUGH THE DOTS

Fluctuations of the background unfortunately set a limit to the accuracy of the test. However, observations of a large number of spot graphs have led us to believe that the error is seldom more than ± 1 second.

RESULTS IN CHILDREN 2 TO 12 YEARS

In Table I are shown the results in 22 children between the ages of 2 and 12 years who had a variety of medical diagnoses, both cardiac and non-cardiac. Only 1 (No. 18) had any condition which might have been expected to alter the speed of circulation. This child had rheumatic heart disease and congestive failure which might have caused a slow rate but actually the rate was rather fast.

Radioactive sodium and saccharin were usually injected simultaneously so that one method could serve as a check against the other. This was done by mixing the measured dose of the activated salt solution with $1\frac{1}{2}$ to 2 cc. of a sterile saccharin solution, made by adding 20 grams of saccharin to 25 cc. of distilled water. For the smaller children $1\frac{1}{2}$ cc. was used and 2 cc. for the larger ones. As seen in Table I there is good correlation between the saccharin and sodium time in some cases and

very poor correlation in others. Where the correlation was poor, it was our impression that the sodium time represented the truer value. It is sometimes difficult for an apprehensive child, even if eager to cooperate, to time a taste end-point accurately. Occasionally a child would be too eager and speak too soon, or on the other hand he might be slow to appreciate the taste. These are single determinations; if the child had been accustomed to the procedure by previous tests, better correlation might have resulted.

In general, the circulation rates in this group correspond with those of older children previously reported. Blumgart & Weiss (2), in their original work with the active deposit of radon, tested the rates of 22 children and young adults ranging in age from 15 to 29 years. The results averaged 17 seconds, varying from 12 to 23 seconds. Tarr, Oppenheimer, and Sager (5) measured the arm to tongue time with decholin in 15 children between the ages of 10 and 14 years. In 8 of these children with normal or well-compensated rheumatic heart disease, the rate ranged from 10 to 17.5 seconds. Averbuck and Friedman (6) de-

TABLE I
Velocity of blood flow in children aged 2 to 12 years

Number	Diagnosis	Age	Weight	Height	Circulation time	
					Saccharin	Radioactive sodium
		<i>years</i>	<i>pounds</i>	<i>inches</i>	<i>seconds</i>	<i>seconds</i>
1	Patent ductus arteriosus	11	84		9	10
2	Rheumatic fever—normal heart	9	61		8	14
3	Rheumatic fever—normal heart	11	61		15	16(± 2)*
4	Rheumatic fever—suspect	10	66		5	11
5	"Grippe"	9	68		9	8
6	Nephritis	10	70	52	6	17(-2)*
7	Splenomegaly	10	70	57	13	14(± 1.5)*
8	Celiac disease	5	29	35		8
9	Ulcerative colitis	11	77	55	7	14
10	Patent ductus arteriosus	8	43		9	7
11	Chorea	9	50	46	14	9
12	Rheumatic heart disease	8	52		3	6
13	Rheumatic fever—normal heart	11	92	60	?	10
14	Rheumatic heart disease	7	45	44	12	13
15	Rheumatic heart disease	11	75	56	12	17
16	Rheumatic heart disease	12	75	60	11	11
17	Rheumatic heart disease—Rheumatic fever	11	70	55	16	13
18	Rheumatic heart disease—decompensated	9	43		?	5
19	Rheumatic heart disease	6	35			10
20	Pyelonephritis	3	30	37		7
21	Pyelonephritis	3	28	37		8

Range 5 to 17 seconds

Average 11 seconds

* Possible correction shown in parentheses. All other determinations have a possible error of less than ± 1 second.

TABLE II
Velocity of blood flow in infants aged 0 to 2 years

Num- ber	Diagnosis	Age	Weight	Height	Circulation time radioactive sodium
			<i>pounds</i>	<i>inches</i>	<i>seconds</i>
1	Convalescent pneumonia	6 weeks	7	21	6
2	Convalescent pneumonia-Pyelonephritis	6 weeks	7	22	3*
3	Convalescent pneumonia-Pyelonephritis (Same as No. 2, one week later)	7 weeks	7	22	6
4	Convalescent pneumonia	4 months	10	23	9*
5	Chronic nutritional disorder	9 months	9	22	6*
6	Celiac disease	20 months	16	30	12
7	Nutritional anemia	15 months	19		5
8	Chronic nutritional disorder	16 months	16	28	7
9	?Scurvy	18 months	19	32	4
10	Upper respiratory infection	22 months	24		11
11	Convalescent pneumonia	13 months	19	30	10
12	Convalescent pneumonia	13 months	19	30	6
13	Pyelonephritis	6 months	18	28	10
14	Cretinism	6 months	11	23	6
Range 3 to 12 seconds		Average 7 seconds			

* Injection into ante-cubital vein (all others into small vein on back of hand).

terminated the rates of 100 normal children, from 8 to 16 years, using saccharin. The average time was 8.6 seconds with a range of 5 to 13.5 seconds.

In the present group of 22 children between 2 and 12 years, the rates determined by radioactive sodium average 11 seconds, with a range of 5 to 17 seconds.

RESULTS IN INFANTS YOUNGER THAN 2 YEARS

In infants, there are technical difficulties which complicate the procedure. In the first place, the ability to make a clean rapid intravenous injection is obviously essential and often difficult. Usually a small vein on the back of the hand was selected for the injection. Care had to be taken not to introduce any radioactive saline into the circulation prematurely, and then to inject the whole dose as quickly as possible.

Secondly, the infants were, as could be expected, actively resenting being held in place and being pricked with a needle. Since there was no satisfactory way of avoiding the factors of struggling and crying, we attempted to determine how much influence these factors may have had. In 2 normal adults, rates were determined by the saccharin method, first at rest, then with glottis closed after a deep inspiration, and finally after exercise (Table III). The second step in this experiment was an attempt to simulate the physiology of crying by increasing the intrathoracic

TABLE III
Circulation rates in 2 normal adults at rest, with increased intrathoracic pressure, and after exercise

	J.P.H.	R.A.R.
Resting	16 seconds	18 seconds
Deep breath (Glottis closed)	22 seconds	23 seconds
After exercise	14 seconds	12 seconds

pressure. This tends to slow the rate and to counteract, therefore, the effect of the exercise. Cannon, Lucia, and Benson (7) have also shown that the increased rate resulting from exercise is not very appreciable. It seems reasonable to conclude, therefore, that the effect of struggling and emotional excitement was slight.

In the group of infants, there were none who had any condition which would be expected to influence the circulation rate. As shown in Table II, there were 14 infants between 6 weeks and 22 months of age. Their sodium rates varied between 3 and 11 seconds, with an average of 7 seconds.

SUMMARY

The velocity of the blood flow in young infants and children has been measured by determining the time elapsed between the injection of radioactive sodium into one arm and its arrival in the

opposite hand. The latter has been signaled by a Geiger counter.

By this method the rate in 22 children between 2 and 12 years was found to average 11 seconds, with a range of 5 to 17 seconds. The rate of 14 infants between 6 weeks and 22 months of age averaged 7 seconds, with a range of 3 to 12 seconds.

BIBLIOGRAPHY

1. Robb, G. P., and Weiss, S., A method for measurement of velocity of pulmonary and peripheral venous blood flow in man. *Am. Heart J.*, 1933, 8, 650.
2. Blumgart, H. L., and Weiss, S., Studies on the velocity of blood flow. *J. Clin. Invest.*, 1927, 4, 15.
3. Warren, S.: Personal communication.
4. Hamilton, J. G., and Stone, R. S., The intravenous and intraduodenal administration of radioactive sodium. *Radiology*, 1937, 28, 178.
5. Tarr, L., Oppenheimer, B. S., and Sager, R. V., The circulation time in various clinical conditions determined by the use of sodium dehydrocholate. *Am. Heart J.*, 1933, 8, 766.
6. Averbuck, S. H., and Friedman, W., Circulation time in normal children. *Am. J. Dis. Child.*, 1935, 49, 361.
7. Cannon, E. F., Lucia, S. P., and Benson, E. H., Circulation time under conditions of work and rest in subjects with normal and abnormal hearts. *Proc. Soc. Exper. Biol. and Med.*, 1939, 42, 237.

PROCEEDINGS OF THE THIRTY-FOURTH ANNUAL MEETING OF
THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION
HELD IN ATLANTIC CITY, N. J., MAY 4, 1942

READ BEFORE THE SCIENTIFIC SESSION

PRESIDENTIAL ADDRESS

By WILLIAM DOCK

During recent years this society has received some very pungent criticism from distinguished past presidents who felt we were not getting the best new members, and that our journal was accepting papers not up to the standards of former years. Our programs, arbitrarily selected by one man, remain subject to constant criticism from members active and emeritus. If we followed the spirit of the times, we would install a full-time salaried editor and secretary, such as now control many national, state, and special medical societies, and hope to benefit by the alleged efficiency of a dictatorship.

It would be well if every faculty member in our universities, every industrialist, and every physician would read, and each year reread, the address on "Academic Freedom in Germany" given by Helmholtz, who was distinguished alike as physician, physiologist, and physicist. It was written at the time when German universities were in their glory and attracted most of the physicians, physicists, and chemists who built up American science between 1870 and 1910. Helmholtz traced the greatness of Germany to the universities, whose students and faculty enjoyed greater freedom than has ever been granted in French, English, or American institutions. The full-time life-tenure dean and college president were not only unknown but were inconceivable to Virchow, Cohnheim, Emil Fischer, and Friedrich Mueller. Their deans, secretaries and other faculty officials were simply colleagues elected for terms of one to three years, a custom wisely followed by this society. This system survived fruitfully until it was abolished by the Nazis, and Helmholtz, who predicted how easily the German schools might be robbed of the freedom which was their proud heritage and chief asset, realized that, once lost, academic freedom probably could never be regained.

It is my hope that this society will not seek efficiency in a benevolent dictatorship, but that its members will manifest a livelier concern in its affairs, voicing criticism of its officers, and offering nominations in competition with those of the nominating committee. Our constitution calls for election of officers by secret ballot, and this certainly does not mean directing the secretary to cast the ballot for a straight ticket each year.

Two years ago your president pointed out the value of conflict in keeping societies alert and productive. We are now engaged in a great struggle which takes its character from the attempt of America and Western Europe to find security and avoid the normal competition and conflict of healthy peaceful civilization. Security, to the politician, meant a legally guaranteed status quo, an

easy way of life. High tariffs, restricted immigration, farm subsidies, the forty-hour week, the peace pacts and treaties all were mere paper barriers against poorer and more energetic people. This game of make-believe led to slavery and chaos in most of Europe, and if we seek that sort of security we dare hope for no better fate.

In the academic world, the term security has long been potent, and, as in the political world, it means a guaranteed easy way of life. It has been held that a life-term appointment would attract to teaching those who so valued security that they would accept lower salaries than they could earn elsewhere. But even universities will not pay our salaries if we become physically disabled, and so long as a physician keeps his health, what security can the university offer him? If he has good judgment and training, is energetic, imaginative, and interested in his work, he will always find patients, institutions, and industrial concerns clamoring for him. Academic security in these days of changing values of money can scarcely attract such men, and it is difficult to see how the university gains by offering security to the mediocre or to those who have become historic monuments. While competent men will not become anatomists, bacteriologists, philosophers or professors of language without the promise of academic security, physicians, physicists, chemists, and engineers who are useful in the university can usually earn a much larger income in practice or in industry. At most, such men need only a sabbatical year for the transition to private life.

It has become quite clear that even in fields which change as slowly as the art of war, the highest effectiveness can be attained only if men reach responsible positions by the age of 35, and full command before 50. Most officers must be retired between the ages of 35 and 55 in order to effect this gravitation of responsibility into the most capable hands. The art of medicine is changing much more rapidly, as is obvious if one compares the medical and military discoveries since 1918. In order to effect the most productive utilization of the facilities given us by society for studying disease and training physicians, we must do everything possible to bring out the best efforts of every member of a medical faculty and profit by the most fertile and productive years of good physicians, regardless of their age. Obviously there must be ample opportunity for young men to bring up families while devoting most of their time to teaching and research, but I have seen no evidence that full-time schools were attracting any better or more productive

men than the part-time medical schools with proper salary levels for the younger staff members.

Stagnation is a very serious problem in many medical schools. It can be avoided if all salaried staff members, all heads of special clinics are appointed for terms of not more than five years. Reappointment should not be automatic, but should mean that no more capable person can be found after thorough search. We should encourage or insist on sabbatical or exchange years in other clinics and facilitate the movement of teachers, and especially of departmental executives, from school to school, or from one department to another. It should be neither unusual nor disgraceful for us to rejoin our colleagues in practice after some years or decades in the academic vale. None of us should hesitate to accept responsibility, nor avoid reentering practice when younger men, as capable as ourselves, are available and can bring new energy and a fresh point of view to the staff. Life might not be so snug for us under such a system, but it would be more stimulating and we would aid greatly in keeping medicine preeminent in a sound social system.

The bureaucracy which is a manifestation of the security complex must be combated in our societies and schools, and in government. Why should one face the arduous life in mine or factory if one can be a salaried labor official? Why should one risk the hazards of industry or finance when from a snug office in Washington one might control industry and finance, avoiding blame or loss if things go badly? Why practice or teach medicine if from a full-time position as secretary of a society, administrator of a school or foundation, one can wield great influence? The men who have accepted such positions are charming and capable people, and are anxious to relieve us of the necessity for managing our own affairs. Few of them believe in rotation in office; like labor leaders and the New Deal, they favor long tenure and do not share the poet's fear that "one good custom (or official) should corrupt the world."

By seeking the type of academic freedom Helmholtz praised, by resisting bureaucracy in our societies, cities and nation, by setting a personal example of willingness to accept short-term appointments, we can contribute, as good physicians, to prolonging the useful life of this civilization. We should see that our practice and curricula are abreast of the best facilities made available by science and society. There is no excuse for commanders who do not understand, insist on having, and fully utilize the modern equipment for security, victory, and providing defense with minimal loss of life; nor for leaders of medicine who do not understand and fully exploit all the chemical and physical discoveries, the techniques, therapeutic methods and devices, no matter how complex or novel, which contribute to safety, accuracy, and speed of diagnosis and treatment. The failure of our generation to make the X-ray a familiar and routine instrument for physical diagnosis is the outstanding evidence that medicine, like some military establishments, may for years misunderstand and undervalue instruments of extraordinary merit, and thus physicians come to use the gifts

of science "too little and too late." We must all be on guard against stagnation in our departments and against falling into rigid patterns of thought.

Three years ago your president offered as the solution of our problems picking executives and teaching personnel of great talent and "contagious fire." But even this is not enough. There are men, like Cushing, Rosenau, and Hektoen, who are ready to accept and eagerly sought for new positions when they reach the legal retirement age. They are rejuvenated by their new tasks. There are men of extraordinary ability who go into the mental menopause prematurely, in some instances rapidly losing a forced and hectic fire when they achieve the academic goal which they had set for themselves. Never forget that Vesalius, at the age of 30, one year after his revolutionary *de Fabrica* came from the press, became a court physician and never contributed anything thereafter. Many similar, if less dreadful, examples are seen about us today, and we must not only select good men but retire those who are not maintaining the "contagious fires" at a healthy glow. None of us are secure if our profession declines, all must give much and risk much to achieve any worthy goal. Today, as in Milton's stormy lifetime, "the immortal garland is to be run for, not without dust and heat." May we neither "slink out of the race" nor seek to bury ourselves in a "fugitive and cloistered" security.

A Study of Calcium Metabolism in Nephrosis. By WILLIAM W. BECKMAN and KENDALL EMERSON, JR. (introduced by Dr. Homer F. Swift), New York, N. Y.

Children with nephrosis characteristically exhibit osteoporosis, hypocalcemia, and hypocalcemia. A detailed study of calcium metabolism in these patients has shown that there is an excessive loss both of this element and of phosphorus from the gastro-intestinal tract, which leads to insufficient retention for the ordinary calcium requirements of growing children. The mere addition of calcium to the diet served only to increase the fecal excretion without improvement in the balance. Large doses of vitamin D failed to influence absorption from the intestines, in spite of which fact there is no evidence of rickets. Likewise the administration of citrate-sodium citrate mixtures had no effect. On the other hand, when lactose was fed, absorption was considerably enhanced, not only of calcium but also of phosphorus, nitrogen, and potassium. Similar results were obtained with dihydrotachysterol (A. T. 10).

The serum calcium concentration was raised to nearly normal by A. T. 10. In spite of this, no increase in urinary calcium was observed. The elevation of the serum calcium to toxic levels by intravenous administration of the ion, caused only a very slight rise in renal excretion. Acidosis increased the loss of calcium in the feces, but had no effect on the urine.

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abnormalities of sprue. At present, our experiments suggest that the latter possibility is the more likely one.

Studies on the Synthesis of Total Coenzyme from the Oral and Parenteral Administration of Nicotinic Acid and Nicotinamide. By CHARLES L. HOAGLAND and ROBERT E. SHANK (introduced by Thomas M. Rivers), New York, N. Y.

The requirement of the influenza bacillus for coenzyme, the physiologically active form of nicotinic acid, was reported by Lwoff and Lwoff in 1936. Since that time a number of attempts have been made to use this organism in the quantitative determination of coenzyme. These techniques have been limited in that turbidity of test material has contributed large errors to the turbidimetric determination. On the other hand, extraction of coenzyme is tedious, often incomplete, and may result in partial destruction of this labile compound.

We have found that the production of nitrite from nitrate by the influenza bacillus parallels closely its metabolic activity, and, in turn, can be related quantitatively to the coenzyme which the medium contains. The method is simple, reproducible, requiring no preliminary extraction of coenzyme, and can be done on less than 1 cc. of blood. By means of the nitrate reduction technique, it has been possible to study quantitatively the synthesis of coenzyme in the blood of animals and human beings, following the ingestion of nicotinic acid and nicotinamide. A definite and reproducible increase in total coenzyme has been observed in the blood when these coenzyme precursors are given orally. Studies will be presented which link the extent of this increase to the stores of coenzyme in tissues and organ depots.

Vitamin B Complex Studies in Dogs. By PAUL J. FOUTS, Indianapolis, Ind.

Dogs fed a high (41 per cent) casein diet supplemented with thiamine hydrochloride, nicotinic acid, riboflavin, pyridoxine hydrochloride, and either pantothenic acid, or a purified liver extract containing "Filtrate Factor," showed suboptimal growth but appeared normal over long periods of time.

When casein in the diet was reduced to 15 per cent, a deficiency developed, consisting of loss of appetite, loss of weight, moderate to severe anemia, and high incidence of skin ulcers.

Of those examined after death, all showed fatty cirrhotic livers, and many showed peptic ulcers.

Para-aminobenzoic acid, inositol, an eluate of a clay absorption of liver extract, and small amounts of choline did not prevent or cure the deficiency. Large amounts of choline produced temporary improvement in some animals. Powdered liver extract was followed by disappearance of all symptoms and satisfactory gain in weight but more rapid gain in weight was noted in dogs receiving the large amounts of choline in addition to the liver extract.

Insulin Resistance. By JACOB LERMAN, Boston, Mass.

Many diabetic patients receiving insulin for the first time develop local reactions. Such reactions usually disappear after a few injections. The serum from several such patients was found not to have any circulating antibodies to insulin. They did show a small titer of antibodies to bovine and pig serum. The serum of two patients with persistent local reactions to insulin also failed to show antibodies to insulin. They responded to insulin in the usual fashion.

This inability of insulin to produce antibodies was verified in numerous animal experiments. Rabbits and guinea pigs receiving insulin subcutaneously or intraperitoneally failed to develop antibodies, nor did their serum protect a normal animal against a minimal lethal dose of insulin.

Occasionally, a patient receiving insulin becomes resistant to its effects and requires tremendous doses to control the diabetes. One such patient required 500 to 700 units daily. This resistant state was verified by the fact that 10 units of insulin intravenously failed to produce the expected drop in blood sugar. The serum of this patient contained circulating antibodies to insulin to a dilution of 1:200,000 and also gave a positive Prausnitz-Kustner test. Later, when the requirement for insulin dropped to 100 units daily, the titer of antibodies in the serum dropped to 1:800. Two other patients who had required 2,000 to 2,200 units of insulin daily at the peak of their resistance, gradually lost resistance over a 1 to 2 year period. When their requirement returned to the normal level (40 to 50 units daily), no circulating antibodies to insulin were detected.

Consequently it may be concluded that (1) antibodies to insulin are antihormonic, (2) insulin resistance is dependent upon the appearance and concentration in the blood of antibodies to insulin, and (3) the return of normal insulin sensitivity is dependent upon the disappearance of circulating antibodies. Similar fluctuations in antibodies have been observed in humans, such as the appearance of antibodies to cow's milk protein, egg white, and other proteins when infants are first exposed to these foods and the subsequent disappearance of the antibodies, in spite of continued ingestion of such foods.* Likewise, I have observed the disappearance of antibodies to thyroglobulin in rabbits repeatedly injected with thyroglobulin.

The Male Climacteric: Its Physiology, Symptomatology, Diagnosis and Treatment. By CARL G. HELLER and GORDON B. MYERS (introduced by Elmer L. Sevringhaus), Detroit, Mich.

Urine gonadotropic titers of three orchidectomized males and five eunuchoids proved to be as high as that found in oophorectomized and menopausal women. Since this loss of gonadal function in the human male was

* Anderson, A. F., Schloss, O. M., and Myers, C. The intestinal absorption of antigenic protein, *Proc. Soc. Exper. Biol. and Med.*, 1925, 23, 180.

shown to be accompanied by a rise in urine gonadotropic hormone, such assays were used as a diagnostic measure to determine testicular function in a selected group of male patients. Eleven of these, ranging in age from 36 to 65 years, exhibited gonadotropic titers considerably above normal. Some of the titers were as high as those found in castrated males and females.

The symptoms of these eleven patients fell into three general groups: (1) Diminution to absence of libido and sexual potency, which was present in all; (2) vasomotor symptoms present in ten; (3) psychoneurotic symptoms present in all.

Gonadotropic titers and symptomatology were closely followed through a four to five week period of intensive testosterone propionate treatment. Similar observations were made after therapy ceased.

It is concluded that gonadotropic assays can serve as an aid in establishing the diagnosis of the male climacteric, and have been helpful in delineating it as a clinical entity. The male climacteric is an aberrant and pathological accompaniment, whereas the female climacteric is an invariable and physiological accompaniment, of the aging process.

Stimulation of Growth in Pituitary Dwarfs with Chorionic Gonadotropin and Sex Hormones. By WILLARD O. THOMPSON and (by invitation) NORRIS J. HECKEL and RICHARD P. MORRIS, Chicago, Ill.

We have previously demonstrated that chorionic gonadotropin and male sex hormone are the most potent stimulators of growth available at the present time, with the exception of the thyroid hormone in patients with cretinism. The effect of chorionic gonadotropin is the result of stimulation of production of male sex hormone by the interstitial cells of the testis. It therefore produces the same effect on the skeleton as male sex hormone. Because of lack of effective preparations of pituitary growth factor it seemed desirable to observe the influence of these materials on the growth of the skeleton in pituitary dwarfism. When either substance is administered to boys with pituitary dwarfism, the following effects on the skeleton are noted:

Rapid increase in length of the skeleton.

Rapid aging of bone, as determined by the roentgen ray, to such extent that the bone age tends to approach the chronologic age.

Development of the musculature and lengthening of the trunk.

Rapid aging of the facial expression.

These changes are associated with growth of the genitalia and development of other secondary sex characteristics. The influence of male sex hormone is not limited to the skeleton but it appears to be a general growth stimulant. Our observations have been correlated with studies in growth by Burgess, Wetzell, Todd, Hodges, Camp and Ciley, and others.

Alterations in Biological Oxidations in Thyrotoxicosis.

By ROBERT H. WILLIAMS, ENRIQUE EGANA, PAUL ROB-INSON, SAMUEL P. ASPER, JR. and CHARLES H. DUTOIT (introduced by Henry Jackson, Jr.), Boston, Mass.

A number of observations in the past indicate certain interrelationships between the brain, pituitary thyroid, and body cell. For example, removal of the thyroid leads to increased pituitary activity; removal of the pituitary is followed by atrophy of the thyroid; vitamin B deficiency has been observed to cause changes in the metabolic rate and in the histology of the thyroid gland. The question of whether disturbances in the metabolism of the body cell may lead to the development of thyrotoxicosis warrants investigation.

We have been studying various segments of the system of biological oxidations in hyperthyroidism, but this report is concerned chiefly with pyruvic acid metabolism.

In a group of 40 unselected thyrotoxic patients from 7 different hospitals in Boston, the blood pyruvic acid, obtained from the patients in a resting and fasting state, was definitely elevated in the majority of instances. Diphosphothiamine, in combination with magnesium and carboxylase, is necessary for the decarboxylation of pyruvic acid. However, in almost all the 40 patients studied, the blood diphosphothiamine and free thiamine were low. It is also of interest that the protein-bound magnesium of the serum is increased in nearly all thyrotoxic patients.

Thiamine balance studies indicate that in some patients it is difficult to get the blood diphosphothiamine to return to normal. Some of the factors which account for this are (1) increased rate of oxidation; (2) relatively high carbohydrate diet; and (3) polyuria, which is common in thyrotoxic patients. These patients excrete more thiamine in the urine than do other vitamin B deficient patients with the same low thiamine blood level and the same diet. Tests on 2 thyrotoxic patients showed that they could phosphorylate thiamine readily.

To study further the changes in carbohydrate metabolism, a series of tests was performed on a group of 6 thyrotoxic and 6 normal subjects. Fifty grams of glucose were given intravenously and 8 specimens of blood were taken during the succeeding 4 hours. The concomitant changes in the glucose, pyruvate, lactate, thiamine, and diphosphothiamine were noted.

In thyrotoxic subjects, the fasting glucose, pyruvate, and lactate were higher than normal and rose to higher levels following the administration of glucose. After the injection of 5 grams of sodium pyruvate, intravenously, the pyruvate and lactate also rose to much higher levels than in normals and they remained high. Marked variations occurred in the response of thiamine and diphosphothiamine; however, in the thyrotoxic patients these substances remained at a lower level throughout the experiment.

In some cases, intramuscular injections of magnesium tended to suppress the rise in the pyruvate and lactate. Administration of diphosphothiamine, intravenously, also had this effect in some instances.

The cause of the development of these alterations in

the biological oxidations and their relationship to the thyrotoxicosis need further study.

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Chesney, Clawson and Webster, and Marine, Spence and Rosen, and others, have observed marked hyperplasia in the thyroids of rabbits fed a cabbage diet. Suk has reported large nodular goiters occurring endemically in a community where cabbage is a principal dietary item. The goitrogenic factor in cabbage has been reported to be certain cyanide compounds found in cabbage and other members of cruciferae family. Subsequently goiters have been produced in experimental animals treated with sodium cyanide, potassium cyanide and methyl cyanide. Robinson and O'Hare, and Barker have reported the development of goiters in hypertensive patients being treated with sodium or potassium thiocyanate.

We are reporting certain metabolic studies and the thyroid histology of one patient who developed a goiter after one year's treatment with potassium thiocyanate administered in treatment of hypertension. The blood cyanate during the period of treatment varied between 3.8 mgm. per cent and 8.9 mgm. per cent. While following the prescribed regimen, the patient improved symptomatically and the blood pressure fell from 220/130 to 140/100 mm. Hg. However, after taking the thiocyanate for one year, the patient complained of swelling in the neck. The swelling was found to be a large goiter over which a loud bruit could be heard. The gland was estimated to weigh about 180 grams. A definite bilateral lid lag and exophthalmos were present. The basal metabolic rate was minus 17. Blood plasma iodine was at the level of myxedema. A biopsy taken from the gland, which at operation was very vascular, disclosed extreme hyperplasia with architecture resembling papillary cystadenoma. The cyanate therapy was stopped, and one month later the thyroid was of normal size and the basal metabolic rate and blood plasma iodine had returned to normal levels.

We feel that the paradoxical findings in this case, *i.e.*, the extreme hyperplasia in the gland, but laboratory signs of hypothyroidism, are of interest and may be of fundamental importance in interpreting thyroid physiology.

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however, in the percentage composition of the 17-KS excretion. Determination of the α and β -steroids disclosed that the latter constituted less than 15 per cent of the total 17-KS excreted by the patients with adrenocortical hyperplasia, whereas in the virilizing adrenocortical tumors the β -steroid fraction accounted for more than half of the total 17-KS excreted.

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A method has been devised for measuring the digestion of suitable solid food substances anywhere in the human gastro-intestinal tract. The substance to be tested is placed in a fenestrated metal cylinder that is housed in an outer cylinder which protects the contained material from the digestive juices. The apparatus is introduced to any desired portion of the gastro-intestinal tract by intubation. At the desired moment, the fenestrated cylinder is partially ejected from its housing by air pressure, thus exposing the test substance to the intestinal juice. The amount of material lost by digestion in a measured period of time is determined chemically.

Data are presented on the digestion of pork heart muscle in 10 normal and 10 achlorhydric stomachs and at varied levels of the small bowel of 2 normal subjects. The relationship between the time of exposure and digestion of the test material was determined in the normal duodenum. Even though all digestive juice is withdrawn from the duodenum by suction before it reaches the apparatus in the jejunum, the amount of digestion of pork heart muscle in 3 hours is not reduced.

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sence of stomach contractions. 4. The hydrogen ion concentration of the stomach content did not directly influence emptying time.

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Three dogs with pouches of the body of the stomach, the acid secreting area (Cope, Oliver, Charles E. MacMahon, Anders Hagstromer and Richard H. Thompson, Gastric Secretion. I. A new gastric pouch with a non-leaking stoma and an intact nerve supply; description of a two stage technic used on the dog. Arch. Surg., 1940, 40, 717), and two with pouches of the antrum were used. Radioactive sodium, as the chloride in hypo-, iso- or hypertonic solution, was placed in the pouch and the absorption measured by the concentration of radioactivity in the blood. Small but reproducible amounts of sodium were absorbed from the acid secreting area; twice as much was absorbed when the animal was fasting and the pouch was secreting a neutral mucoid secretion as when the pouch was producing acid. The tonicity of the solution made no difference. In the dogs with antral pouches, approximately 100 times as much sodium was absorbed per unit of surface area of mucosa. There was slightly greater absorption from the hypertonic solution but it made no difference whether the animal was fasting or digesting.

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output, as did peripheral vasodilatation or vasoconstriction produced by warming or cooling the body. Greatly increasing peripheral blood flow in the limbs by releasing an arterial occlusion of some duration (reactive hyperemia) caused a considerable increase in cardiac output but of shorter duration and of lesser magnitude than the estimated increase in blood flow in the limbs. The relatively small changes in cardiac output produced by the above procedures were attributed to the buffering effects of the vasomotor system. For example, the presence of a compensatory vasoconstriction elsewhere during reactive hyperemia in the limbs could be demonstrated if the circulation in these limbs were suddenly reoccluded a few seconds after being released. There was then temporarily a rise in arterial pressure, and a reduction in cardiac output, often beyond the levels which had existed prior to the release.

The Relation of Postural Hemodilution to Paroxysmal Dyspnea. By GEORGE A. PERERA and ROBERT W. BERLINER (introduced by Robert F. Loeb), New York, N. Y.

The present study was undertaken to see whether the well-established shifts of body fluid to the blood stream following rest in the horizontal position might be a factor in inducing left-sided heart failure as manifested by paroxysmal dyspnea.

In order to study the degree of hemodilution which may take place in the horizontal position and to correlate these changes with the time of appearance of attacks of paroxysmal dyspnea, observations were made on the serum protein concentration at 2-hourly intervals during the course of 24 hours in 30 normal, ambulatory cardiac, and bed patients. Confirmed by exercise and tilt-table experiments, the results indicated that recumbent rest was associated with a 10 to 15 per cent drop in serum proteins, presumably due to an increase in plasma volume. Supportive evidence of hemodilution was obtained from hematocrit and red blood cell count determinations. Hemodilution was associated with a tendency toward an increasing venous pressure and a reduced vital capacity. No change was noted in the ratio of albumin and globulin.

Diurnal studies of 8 patients with paroxysmal dyspnea showed that the attacks took place at a time when maximal hemodilution was present, *i.e.*, during the early morning hours when the episodes are classically most apt to occur. The attacks were terminated by assuming the erect position and moving about, activities which were associated with a rapid rise in serum protein concentration.

The effect of assuming the horizontal position with its attendant hemodilution is the equivalent of giving a slow but sustained infusion. In support of this are the observations of Caughey and others that infusions in patients with a diminished cardiac reserve may precipitate pulmonary edema. Furthermore, Swank, Porter, and Yoemans have recently shown that increases in blood volume of 50 per cent or more can induce manifestations of left-sided failure in normal dogs.

It is concluded that the increase in plasma volume induced by horizontal bed rest is a possible factor in the production of paroxysmal dyspnea.

Relationship Between Edema and Rate of Peripheral Blood Flow. By DAVID I. ABRAMSON and SIDNEY M. FIERST (introduced by Louis J. Soffer), Cincinnati, Ohio.

The rate of peripheral blood flow was investigated in a series of patients with edematous extremities, using the venous occlusion plethysmographic method. The results varied, depending upon the etiological factors producing the condition.

In three patients, unilateral edema was present as a result of metastasis to regional lymph glands. In two of these, the upper extremity was involved, and in both instances the blood flow to the hand was practically twice as great as that to the normal extremity. In the remaining case, the edema was present in one lower limb, and the involved leg demonstrated a rate of blood flow which also was almost double that in the other.

In two patients, edema of the affected upper extremity was present during the acute stage of hemiplegia. In the one in whom the condition was more marked, the blood flow in the involved hand was almost three times that in the normal one. In the other, no difference in the flow was noted between the two hands.

In four patients, bilateral edema, unassociated with heart failure, was present in the lower extremities; the circulation time in each case being within normal limits. The blood flow in the leg in every instance was significantly increased as compared with the average flow in the control group.

In five patients, bilateral edema of the lower extremities was present as a result of cardiac decompensation. The rate of blood flow in the leg in this group fell within the range of the figures obtained for the control series.

The possible mechanisms responsible for the changes in blood flow in edema are considered.

Renal and Total Circulation in Two Cases of Constrictive Pericarditis. By MILTON LANDOWNE (by invitation), ALF S. ALVING and (by invitation) WRIGHT ADAMS, Chicago, Ill.

The criteria for renal ischemia with "increased efferent arteriolar constriction" were satisfied for each of two patients with proven chronic constrictive pericarditis. The deviation from normal was more marked than that encountered in arterial hypertension although the blood pressures were 134/98 and 84/70. The "effective renal blood flow" ($C_D/\text{hematocrit} = 752$ and 528 cc. per minute per 1.73 m.²) was reduced proportionately more than the cardiac output ($C.I. = 1.84$ and 1.58), the renal fraction being 83 per cent and 65 per cent of its expected value. Glomerular filtration was slightly reduced ($C_I = 120$ and 90 cc. plasma per minute per 1.73 m.²). The plasma "filtration fraction" was, therefore, extremely high (31.7 per cent and 31.9 per cent, increases of six times the

standard deviation (σ) of the mean normal values). Maximal tubular excretion of diodrast was normal ($Tm_D = 46.0$ and 50.5 mgm. I. per minute per 1.73 m.²), and consequently, the "plasma flow per unit of functioning tubular mass" was very low ($C_D/Tm_D = 8.27$ and 5.55 cc. per mgm. I., reductions of 3.6 and 5.5 σ). Plasma from each of these patients, when tested by Dr. I. H. Page upon the denervated rabbit ear, was markedly constricting.

Elevated systemic venous pressure, by increasing resistance to post-glomerular perfusion, may mimic "increased efferent arteriolar resistance." These observations are also compatible with the hypothesis that the kidney participates in a vascular regulation, where, in the presence of a reduced cardiac output, renal efferent arteriolar constriction and a generalized peripheral vasoconstriction are produced to maintain normal blood pressure.

*Search for Renin and Determination of Hypertensin Precursor in Plasma of Normal and Hypertensive Patients.** By LEWIS DEXTER and FLORENCE W. HAYNES (introduced by E. S. Emery, Jr.), Boston, Mass.

The relationship of experimental to human hypertension is of exceeding importance and is as yet unclarified. A search for the presence of renin in the blood of hypertensive patients has therefore been made, together with estimations of hypertensin precursor in normal and hypertensive patients.

Modifications of the direct renin method and of the precursor method of Leloir and his associates were used. Human renin was prepared as described by Braun-Menendez and his associates and their assay method was employed in cats which are 10 to 20 times more sensitive than dogs.

The concentration of hypertensin precursor in human plasma in 11 hypertensive and 8 normal patients was found to be the same, averaging 2.67 and 2.45 cat units per cc. of plasma respectively. This is approximately the same concentration present in dog and beef plasma.

In our hands, the direct renin method is capable of detecting 0.005 cc. (0.4 cat unit) of a solution of human renin added to human plasma. This method eliminates non-specific substances and has the same order of sensitivity as the perfusion methods of isolated organs as described by other workers. No renin was demonstrable by this method in 10 cc. of plasma of 7 patients with severe chronic hypertensive disease, one of whom had malignant hypertension.

On the Pressor Activity of Extracts of Hypertensive Blood. By HENRY A. SCHROEDER and C. CHESTER STOCK (introduced by C. P. Rhoads), New York, N. Y.

An attempt has been made to find whether the blood of patients exhibiting arterial hypertension contains

pressor or other abnormal substances not present in normal individuals.

Concentrates were made from the acidified alcoholic filtrates of 50 large samples (120 to 400 cc.) of arterial blood so that 1 cc. equalled 20 cc. of original blood. They were taken from 38 patients of whom 24 had hypertension and 14 did not. Certain differences were found in the extracts taken from the two groups.

A. Picrates of these extracts were formed and the intensity of color was measured according to Richter's method. A color scale was used of concentrations of iso-amyl amine picrate. In 32 samples of hypertensive blood, the range ran from 1 to more than 10γ per cc. of original blood, the average being 5.6γ . In half, it was more than 4. In 14 samples of normal blood, it varied from 6.7 to 0γ per cc. (average 2.2), in only 3 being more than 4. Extracts of plasma gave more color than did those of cells.

B. The extracts from 18 of 20 hypertensive patients caused pressor responses in rats on intravenous injection, lasting 15 to 30 minutes; and in only 2 of 14 normal subjects. That portion of the extracts occasioning pressor effects was insoluble in petroleum ether and when alkaline was soluble in toluene. It was partially purified by chromatographic adsorption, and disappeared after treatment with amine oxidase. Toluene extracts containing picrates made from 12 samples of hypertensive blood were likewise pressor; those made from 5 samples of normal blood were not.

In 15 hypertensive patients, the extracts contained the pressor material and the picrates together; in normal patients, both were present in only 2 cases. These observations indicate that there are differences between the bloods of hypertensive and of non-hypertensive patients.

Further Studies of the Mechanism of Nephrotoxic Nephritis in Rabbits. By CALVIN F. KAY (introduced by O. H. Perry Pepper), Philadelphia, Pa.

Nephrotoxic nephritis, produced by the injection of anti-renal sera, has been studied extensively because of its remarkable clinical and pathologic similarity to human glomerulonephritis. The virtue of the disease as an experimental medium has, however, been dulled by the lack of analogy between the supposed mechanism of the disease—an attack by injected antibodies directly upon the antigen inherent in the kidneys of the recipient—and any conceivable mechanism in human nephritis.

Two years ago it was suggested (Kay) that immunologic affinity binds nephrotoxic duck protein to the kidneys of the injected rabbit, but that nephritis ensues only when the nephrotoxin-kidney combination is attacked by anti-duck protein antibodies formed by the rabbit. The latent period between injection and the onset of the nephritis was found to be related to the rate of formation of anti-duck antibodies by the rabbit. Inhibition of antibody formation by the use of x-ray prevented the development of the nephritis.

The validity of this concept appears to be proven by the demonstration in the present experiments, fully con-

*The expense of this investigation was defrayed in part by grants from the Armour Laboratories and from the Markle Foundation.

trolled, that nephritis is readily induced in rabbits exposed to x-ray and injected with nephrotoxic serum by the passive transfer of antibodies to duck serum. The concept is interestingly analogous to the hypothesis that human glomerulonephritis develops as the result of the interaction of antibodies with an antigen formed as the result of the action of some product of the streptococcus upon the kidney.

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There is strong evidence that this substance is actually PAB since it is rapidly destroyed by a suspension of soil bacilli which have been specifically adapted to oxidize PAB, and is not attacked by the basal inactivated cells of this bacillus. Furthermore, the inhibitor produced by the pneumococci specifically activates the PAB oxidizing enzymes of the soil bacilli grown in its presence.

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and retarded. When put into the duodenum, the sodium salts were absorbed rapidly and completely. Sulfanilamide was absorbed more rapidly and more completely than the other three drugs, when given by mouth or into the duodenum. Absorption of this drug also was retarded when it was given after a meal.

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by a mild respiratory tract infection, nuchal rigidity, photophobia, mental confusion or somnolence, lasting from 3 to 9 days. Laboratory data were at variance with the usual neurotropic virus diseases in that polymorphonuclear leukocytosis was uniformly present in the blood and spinal fluid. Furthermore, immunologic tests and animal inoculation with spinal fluid and blood serum, performed by Doctors Joseph Smadel and Charles Armstrong, gave entirely negative results, which rules out lymphocytic choriomeningitis, equine encephalitis, St. Louis encephalitis, Japanese B. encephalitis and louping ill. Viennese (von Economo) encephalitis and atypical poliomyelitis were also considered in clinical differentiation but could not be tested for, for the want of helpful tests. The 4 cases we dealt with therefore appear to be ones of a still unclassified form of so-called aseptic meningitis or meningoencephalitis.

German Measles as an Incitant of Rheumatic Fever. By MARK P. SCHULTZ, Washington, D. C.

Reports in the British military medical literature recently have expressed concern over the possible association of carditis with German measles. In the study of a recent epidemic, we have found that although arthritis is frequently associated with such infection, carditis appears to be a manifestation of rheumatic fever activity, apparently provoked by the measles infection. This study comprised the use of throat cultures and antistreptolysin titrations of serum to evaluate the possibility of hemolytic streptococcus infection being responsible.

*Studies of Biotin Metabolism in Man.** By THEODORE W. OPPEL (introduced by David P. Barr), New York, N. Y.

Biotin, the most recently purified member of the vitamin B complex, is used by many microorganisms and at least several species of animals. Chicks become deficient if they receive less than 7 to 10 gamma of biotin per 100 grams of ration, but rats cannot be made deficient by a low biotin diet. They must be fed large quantities of egg white. This contains a material called avidin which prevents the absorption of the vitamin.

The metabolism of biotin in human subjects has been studied. Ordinary diets contain from 30 to 65 gamma, and alterations in the amount of biotin in the food were accompanied by corresponding alterations in the urine. The amount in the urine, however, often exceeded the amount in the diet, and even when no food was consumed the urine continued to contain quantities of biotin that were relatively normal. This urinary biotin was provided by synthesis of the vitamin by the bacteria in the intestinal tract, for the stools invariably were found to contain more biotin than the diet. From 3 to 6 times as much biotin was eliminated in the urine and stools as the diet contained.

Apparently in man there is a relatively constant level of biotin excretion in the urine, based on biotin synthesis

* This investigation has been aided by a grant from the Josiah Macy, Jr. Foundation.

by intestinal bacteria and, superimposed on this, sudden variations occur due to changes in the biotin content of the diet. The quantity of biotin provided by bacterial synthesis seems to be adequate for the needs of man, and human subjects probably do not require any biotin in their food.

Studies with Radioactive Di-Azo Dyes. By FRANCIS D. MOORE* and LESTER H. TOBIN (introduced by Joseph C. Aub), Boston, Mass.

Radioactive analogues of two colloidal dyes (trypan blue and Evans blue or T-1824) have been synthesized. The synthesis is not time-consuming and produces a di-brom radioactive dye of known chemical structure and adequate radioactivity for studies in the intact animal or excised tissue. Chemical and biological properties of the radioactive dyes are those of their non-radioactive counterparts, and include accumulation in areas of increased capillary permeability, such as abscesses and tumors.

Potential use as a means of localizing abscesses has been studied in rabbits. Peripheral abscesses were detectable in all instances, the dye being given intravenously and the animal "scanned" with an externally-placed Geiger counter. Abscesses on the abdomen were less reliably diagnosed, 77 per cent giving positive results.

Possible usefulness as a means of internally radiating tumors has been investigated in mice. Ten per cent of the tumors accumulated the dye in concentrations equal to or greater than the kidneys and spleen. Only a very small group accumulated the dye in concentrations greater than the liver. Such radioactive colloids might be useful as internal radiation therapy if the tumor were widespread and of high sensitivity. Tumors undergoing haemorrhagic necrosis accumulate significant radioactivity in the haemorrhagic fluid, suggesting the usefulness of coincident therapy with agents increasing the permeability of capillaries within the tumor.

The Prevention of Electrocardiographic Evidences of Myocardial Anoxemia by Chemical Means. By SAMUEL PROGER and (by invitation) MARK AISNER, and RAYMOND B. SQUIRES, Boston, Mass.

Certain chemical substances of the C₄ dicarboxylic acid group have been shown to promote tissue oxidation catalytically.

Under conditions of decreased oxygen tension, the catalytic effects *in vitro* of succinic, fumaric, malic, and oxaloacetic acids are even greater than under conditions of normal or high oxygen tensions; in some cases, in a 10 per cent oxygen environment, the oxygen uptake of minced heart muscle of a dog is increased by more than 100 per cent by these substances.

In intact anesthetized dogs, and under the conditions of our experiments, certain electrocardiographic changes which occur in the presence of 10 per cent anoxemia are sometimes apparently prevented by the previous intravenous administration of sodium succinate.

* Fellow in Medicine, National Research Council.

The Effect of Varying Blood Sugar Levels on the Electroencephalogram in the Normal Adult During Normal Breathing and Hyperventilation. By JACOB E. FINE-SINGER and (by invitation) M. A. B. BRAZIER and ROBERT S. SCHWAN, Boston, Mass.

The results of previous workers with very small numbers of subjects have shown that there is a great individual variation in the electroencephalographic pattern. The present study deals with quantitative changes in blood sugar level, respiratory volume, electroencephalographic rhythm, and their intercorrelations in a series of 45 normal subjects. Repeated electroencephalographic tracings were obtained on each subject during a 3-minute period of normal breathing, followed by a 3-minute period of controlled and measured hyperventilation. Blood sugar determinations were made after each test.

The following results were obtained:

1. Normal ventilation.

(a) No delta activity was observed during normal breathing at any blood sugar level.

2. Hyperventilation.

(a) In 22 per cent of the subjects, no delta wave activity at fasting blood sugar levels was observed. Delta wave activity could be elicited after the administration of insulin.

(b) Fifty-three per cent of the subjects showed delta waves at fasting blood sugar levels, but not after the ingestion of glucose.

(c) The remaining 25 per cent showed the presence of delta activity at all blood sugar levels, even after the ingestion of glucose.

3. In some subjects, low blood sugar levels produce a slowing of the alpha rhythm during normal breathing.

4. In subjects in whom the ingestion of glucose inhibits the production of delta activity on hyperventilation for 3 minutes, the critical blood sugar level is approximately 130 mgm. per 100 cc.

5. In tests in which the blood sugar level was the same, the depth of respiration was the deciding factor in determining the amount of delta activity present.

6. Since the frequency of alpha rhythm and the presence of delta waves are now used in assessing normal electroencephalographic records, it is essential that all tests be carried out at non-fasting blood sugar levels.

Central Sympathetic Paralysis. By EUGENE A. STEAD, JR. and (by invitation) JOHN ROMANO and RICHARD V. EBERT, Boston, Mass.

The disturbances in function of the sympathetic nervous system, resulting from lesions in the medulla produced by thrombosis of the posterior inferior cerebellar artery, have been studied in 6 patients. This central sympathetic paralysis differs strikingly from that produced by pre- or post-ganglionic sympathectomy. Instead of the complete loss of sympathetic function which peripheral sympathectomy produces, these patients showed selective impairment. Sweating and vasoconstriction in response to cool-

ing the body were markedly impaired. Other functions, such as vasodilation from heating the body and vasoconstriction in response to sensory stimuli or a deep inspiration, were not affected.

The mechanism which inhibits vasoconstriction when the body is cooled, without interfering with vasodilation when the body is heated, is of both theoretical and practical importance. The patient with Raynaud's disease tends to improve after either pre- or post-ganglionic sympathectomy, because cooling the body no longer reduces the blood flow to the extremities. In a cool room, however, he has the severe disadvantage that heating the body by wearing warm clothes no longer increases the blood flow to the extremities. In the sympathetic paralysis produced by lesions in the lateral medullary area, this disadvantage does not exist.

Experiments were performed which showed that vasoconstriction is an active process, while vasodilation is a passive one caused by inhibition of vasoconstriction. This fact explains why, in this type of central sympathetic paralysis, vasoconstriction on cooling the body is incomplete, whereas vasodilation is unaffected. The lesion in the medulla destroys the main uncrossed vasoconstrictor tract, but partial vasoconstriction still occurs either because the lesion in the medulla does not destroy all the uncrossed descending sympathetic fibers or because there are some fibers from the opposite side which cross below the lesion. Inhibition of vasoconstriction, however, results in normal vasodilation, because while there is not enough sympathetic activity to produce full vasoconstriction, there is enough sympathetic function to prevent the marked increase in tone of the blood vessels which occurs after pre- or post-ganglionic sympathectomy.

Cutaneous and Visceral Pain Sensitivity in Normal Subjects. By WILLIAM P. CHAPMAN (by invitation) and CHESTER M. JONES, Boston, Mass.

One hundred and eighty normal subjects of various ages, races, occupations, and emotional types have been studied for their perception and reaction to cutaneous pain, by a modification of the heat-radiation apparatus of Wolff, Hardy, and Goodell. Thirty of these individuals were tested for visceral pain sensitivity by distending the lower end of the esophagus with a balloon. The perception of the pain threshold for cutaneous pain has been taken as a beginning, sharp jab sensation, and the "reaction" to pain as the first motor response to the pain stimulus. The only reliable end-point for visceral sensitivity appeared to be the lowest stimulus which produced a substernal sensation of beginning fullness. A correlation of this visceral sensory threshold was made with the cutaneous pain threshold in those subjects tested by both procedures.

The results of the cutaneous pain tests indicate a wide range of sensitivity as regards variation in pain threshold and reaction to pain. Pain sensitivity appeared to decrease with age and to vary with race. A group of individuals of Nordic stock were less sensitive than similar groups of Negroes, Italians, and Russian Jews. There

was a significant correlation between the cutaneous pain and visceral sensory thresholds.

The Effect of Cigarette Smoking on the Peripheral Blood Flow. By WILLIS F. EVANS (by invitation) and HAROLD J. STEWART, New York, N. Y.

In recent years, there has been added interest in the effects of smoking tobacco on the human organisms. This has to some extent paralleled the increase in interest in the peripheral circulation. There are in the literature data relating to the effect of smoking cigarettes on the peripheral blood flow, blood pressure, and heart rate. The inferences about peripheral blood flow have been made from volume changes in a digit or extremity by modified plethysmographs or from local change in temperature. We have now measured the average peripheral blood flow for the whole body surface in cc. per M² per minute, by a modification of the method of Hardy and Soderstrom, in the course of which we secured not only temperatures of various areas of the body but also the average surface temperature; in addition, we have recorded blood pressure, pulse rate, and basal metabolic rate for correlation with the changes in peripheral blood flow.

Observations were made of 10 normal male subjects. All observations were made in the morning while the subjects were in a basal metabolic state, data being recorded before, during, and after smoking. Each subject smoked on successive days regular or standard cigarettes, so-called commercial "denicotinized" cigarettes, and cigarettes made of cornsilk. In addition, 4 observations were made of 3 subjects to secure data about "fully denicotinized" cigarettes. The following effects were observed: regardless of the type of cigarette smoked, whether "regular," "denicotinized," "fully denicotinized," or cornsilk, there occurred, in all except 4 instances, a moderate fall in peripheral blood flow, that is to say, the average amount of blood allotted to the periphery was decreased. There occurred rise in blood pressure and in pulse rate, fall in temperature of the extremities and in the average surface temperature, and increase in rectal temperature of essentially the same magnitude and duration regardless of the type of cigarette which was smoked. Nicotine has been credited in the previous studies with inducing these effects. Since changes of the same magnitude occurred from the smoking of commercial denicotinized cigarettes, from those fully denicotinized, and from those made of cornsilk, it appears that it is not nicotine but some other unknown substance which is responsible.

The Relationship of the Coagulation Defect in Hemophilia to a Plasma Proteolytic Enzyme. By HENRY J. TAGNON, CHARLES S. DAVIDSON, and F. H. L. TAYLOR (introduced by George R. Minot), Boston, Mass.

By shaking oxalated or citrated platelet free human plasma with chloroform, a clot is produced which redissolves entirely in the subsequent 5 to 10 days; when dissolution is complete, the preparation, called chloroform

plasma, contains an active proteolytic enzyme, responsible for the dissolution of the clot, and for the proteolytic behavior of subsequent preparations. This enzyme is associated with the globulin fraction of the chloroform plasma from which it can be separated by precipitation at pH 6 or by dialysis against water. The proteolytic activity of this preparation is evidenced by its digestive action on gelatin and casein as well as on fibrinogen and fibrin, in the absence of bacterial contamination.

Previous investigations have shown that the coagulation defect in hemophilia is due to the deficiency of a cell free plasma factor associated with the globulin fraction obtained by precipitation of blood plasma at pH 6 or by dialysis. When hemophilic plasma is shaken with chloroform, the clot formed redissolves in a longer time than that of normal plasma. Also, when the dissolution is complete, the enzymic activity of preparations from hemophilic plasma when tested on fibrinogen and fibrin and compared to preparations from normal plasma is markedly lower.

These results indicate that, in hemophilia, the deficiency of a factor associated with the globulin fraction of the plasma ("globulin substance") is paralleled by a deficiency in proteolytic activity associated with the globulin fraction of chloroform plasma.

Observations on the Effect of Promin on the Blood of Tuberculous Patients. B. E. HALL, H. C. HINSHAW, and KARL PFUETZE (introduced by H. Z. Giffin), Rochester, Minn.

In tuberculosis, treatment with sodium p,p'-diaminodiphenylsulfone-N,N'-didextrose sulfonate ("promin") necessitates administration of the drug over long periods and affords the opportunity of studying the delayed as well as the immediate effects of this substance. This is in contrast to most diseases in which chemotherapeutic agents of sulfonamide type are administered for only short periods. Eighty-one cases are included in this study. Of these, seventy-six were cases of pulmonary tuberculosis, three were of renal tuberculosis, one was of tuberculosis of bone, and one was of tuberculous meningitis. Of the cases of pulmonary tuberculosis, hematologic studies extended over a period of from four to nine months in sixty-seven cases and from three to three and a half months in five cases.

The principal effect of promin on the blood was the development of anemia as a result of excessive destruction of blood. It occurred in all cases and was used as one of the criteria for gauging doses. Moderately severe acute anemia was observed during the administration of comparatively large doses of the drug; on smaller doses, chronic anemia was noted. In one case, extremely acute hemolytic anemia developed with alarming rapidity during the administration of small doses of promin over a period of three days.

Leukopenia with neutropenia of moderate degree was observed in four cases and agranulocytosis associated with ulcerative lesions in the throat occurred in one case.

A Report of Clinical Studies with the Synthetic Dicoumarin 3,3'-Methylenebis (4-hydroxycoumarin). By OVID O. MEYER and (by invitation) JAMES B. BINGHAM, Madison, Wis.

Dr. Karl Paul Link and his associates of the Wisconsin Agricultural Experiment Station have reported the isolation of the agent in spoiled sweet clover responsible for the hemorrhagic disease of cattle, and the subsequent successful synthesis of a dicoumarin which is biologically identical.

During the past 18 months, this synthesized substance, 3,3'-methylenebis (4-hydroxycoumarin) has been employed in animal experiments and clinical studies. Published reports have described the experiences in dogs, the significant pathologic changes, which included dilatation of smaller vessels, the lack of toxicity attendant upon its use in reasonable dosage and the efficacy in prolonging the prothrombin time and coagulation time when administered orally, or intravenously in the form of the disodium salt.

The present report deals with additional clinical experiences in cases with thromboses, peripheral vascular disease, subacute bacterial endocarditis, and other conditions. A total of 98 cases have been treated. The present dosage, the indications and contraindications, the advantages over heparin, and the hazards associated with the use of this dicoumarin will be discussed. Briefly it may be stated that a satisfactory oral dose for most patients (there is considerable individual variation in response) is 5 mgm. per kgm., followed by daily doses of 1.5 mgm. per kgm. To date, it has not been demonstrated that any clinical condition contraindicates the use of the drug if local bleeding, hemorrhagic states, and probably advanced hepatic disease be excluded. The advantages of the material over heparin include the ready availability and low cost, the two successful routes of administration, and the sustained effect. The chief disadvantage observed to date is the protracted effect after the drug is stopped. The coagulation time can be reduced temporarily with fresh blood transfusions, but not with vitamin K administration.

The Dicoumarin-3,3' Methylenebis 4 Hydroxycoumarin. Its Pharmacological and Therapeutic Action in Man. By IRVING S. WRIGHT and (by invitation) ANDREW PRANDONI, New York, N. Y.

This paper presents data obtained from the study of thirty patients who have received this substance under well controlled conditions. Data on bleeding and coagulation times, prothrombin studies, clot retraction, liver and kidney function tests, gastric analysis, and other laboratory and clinical observations will be presented.

This dicoumarin produces prolongation of the clotting and prothrombin times. The mechanism of action and the clinical effects and complications will be discussed. Therapeutic indications will be evaluated. Recommended dosage, methods of administration and of producing cessation of action when desired will be outlined.

Report of Experimental and Clinical Studies with Dicoumarin. By E. V. ALLEN and (by invitation) J. L. BOLLMAN and N. W. BARKER, Rochester, Minn.

Experimental and clinical studies indicate that dicoumarin administered orally increases greatly the prothrombin time of the blood and increases less markedly the extravascular coagulation time of the blood. Clinical and experimental studies indicate that when the dosage of the drug is carefully regulated, no harm results, as no morphologic or physiologic effects upon the blood, liver, kidneys, and other vital structures have been noted, with the exception of the effect on prothrombin and coagulation time. Two hundred milligrams given on two or three successive days usually prolongs the prothrombin time greatly for from seven to ten days, after a latent period of twenty-four to thirty-six hours after the original dose. When large amounts of the drug are given to animals, fatal hemorrhage may occur. The drug has been administered to approximately seventy-five patients without harmful effects, except for controllable hemorrhage from operative wounds in six instances. The prothrombin time and coagulation time of the blood of both animals and human subjects can be temporarily restored to normal by the transfusion of fresh blood. Vitamin K is almost entirely ineffective in reducing the prothrombin time when it has been increased by administration of dicoumarin. The preparation has been found to be very useful in experimental studies where it is desired to avoid the intravascular coagulation of the blood. Intravascular thrombosis, such as that which occurs in thrombophlebitis, has not affected any of the treated patients after treatment was begun and pulmonary embolism has been entirely lacking, although many of the cases were those in which the dangers of venous thrombosis and pulmonary embolism were great. The series is, of course, too small to allow a statement relative to the use of this drug for prophylaxis against intravascular thrombosis. It appears that the use of dicoumarin may replace the use of heparin. Obvious advantages are effectiveness by oral administration, and cheapness. Since dicoumarin has been used clinically, the authors have almost entirely ceased the use of heparin.

READ BY TITLE

Studies on the Administration of Normal Human Plasma Preserved in the Liquid State. By L. R. NEWHOUSER, D. B. KENDRICK, and EUGENE L. LOZNER (introduced by Clark W. Heath, Boston), Washington, D. C.

The results of over 600 administrations of normal human plasma, preserved in the liquid state, to over 300 patients have been analyzed. This plasma was prepared by a closed system without any filtration. A glass cloth or steel mesh filter was used during the administration. The elapsed time between drawing the donors' blood and administering the plasma varied from 2 weeks to 12 months. The average time was 2½ months. The temperature at which the plasma was preserved varied from 4° to 25° C. The amount administered to any one patient varied from 30 ml. in an infant to 2500 ml. in a

patient with severe peripheral circulatory failure. The average amount administered was 500 ml. The most frequent indications for which plasma was administered were the treatment and prevention of secondary shock and the treatment of hypoproteinemia. In 86 per cent of the administrations, the therapeutic result was beneficial, and in 14 per cent, it was equivocal. In 1 per cent of the administrations, untoward reactions attributable to the plasma occurred. The most frequent of these was urticaria. It may be concluded that normal human plasma may be preserved in the liquid state for periods up to at least 12 months and be administered to patients with safety and benefit.

An Experimental Evaluation of the Intensive Drip and Other Intensive Methods for the Treatment of Syphilis.
By HARRY EAGLE and (by invitation) RALPH B. HOGAN, Baltimore, Md.

The total curative dose of mapharsen in syphilitic rabbits was only slightly affected by wide variations in the frequency of injection, and in the total duration of treatment. On any method of treatment, whether intravenous drip, or intravenous injections repeated several times daily, daily, three times weekly, or weekly, the total amount of arsenical tolerated did, however, increase directly with the duration of treatment. It follows that the margin of safety (chemotherapeutic index) on any treatment schedule may be increased many-fold by suitable prolongation of the treatment period.

In the experimental animal, an intravenous drip over a four day period provided a margin of safety between the effective and toxic levels only one-third to one-fourth that afforded by standard clinic practice of weekly injections over a period of many months. The animal studies did, however, suggest that it may be possible to concentrate treatment safely along more conservative lines, and to administer effective amounts of arsenical within a period of four to ten weeks, with a margin of safety comparable to that afforded by current procedures. Clinical studies in this direction are now in progress.

Familial Mediterranean (Target-Oval Cell) and Familial African (Target-Sickle Cell) Anemias. By WILLIAM DAMESHEK,* Boston, Mass.

Studies of Italian families have revealed several syndromes ranging in severity from Cooley's erythroblastic anemia and the previously described "Target Cell" Anemia to cases with mild hypochromic anemia. Severe cases show splenomegaly and jaundice, milder ones either "hypochromic polycythemia" or mild hypochromia, target and oval erythrocytes, increased hypotonic resistance, basophilic stippling, and refractoriness to iron therapy.

These syndromes, inherited as a Mendelian dominant, present a high incidence of transmission in the offspring. Milder syndromes are inherited from one parent, but the severe Cooley's anemia apparently requires transmission from two mildly affected parents. Mediterraneans with splenomegaly, a cardiac systolic murmur, diminished

hemoglobin, erythrocytosis, refractory hypochromic anemia, hemolytic jaundice, or basophilic stippling should be suspected of a target-cell syndrome. The fundamental inherited abnormality is probably a disturbed hemoglobin metabolism with the resultant production of abnormally thin erythrocytes.

Numerous clinical and hematological resemblances between the essentially Mediterranean target-oval cell syndromes and the essentially African target-sickle cell syndromes suggest a fundamental relationship between them. This is confirmed by transition forms between the two diseases. A large reservoir of flat red cells ("leptocytosis") exists in both racial groups, appearing frequently as either target-oval or target-sickle-cell syndromes.

A New Conception of the Cause of Patency of the Ductus Arteriosus Based upon Experiments on Its Physiology.
By J. ALLEN KENNEDY (introduced by C. Sidney Burwell, Boston), Nashville, Tenn.

There have been many theories of the mechanism of closure of the ductus arteriosus at birth, none of them backed by experimental evidence. Our studies on the physiology of the ductus arteriosus in guinea pigs have resulted in the following:

(1) The mechanism of closure of the ductus arteriosus at birth has two phases:

- (a) An immediate one much like the contraction of a muscular sphincter which functionally closes its lumen, and
- (b) A slower histological change during which the ductus is transformed from a muscular tube to the fibrous connective tissue ligamentum arteriosum.

(2) The ductus arteriosus will close in response to the following:

- (a) Normal breathing.
- (b) Mechanical or electrical stimulation to the ductus.
- (c) Artificial inflation of the lungs through a tracheal cannula with either air or oxygen.
- (d) Injection of adrenalin.
- (e) Massage of the carotid sinus.

Closure of the ductus is not a neurological mechanism. Under the proper condition the ductus may be closed and opened many times.

Based on these experiments we have arrived at a new conception of the cause of patency of the ductus arteriosus. Instead of being a developmental anomaly or abnormality, it may be due to failure of the normal physiological process of closure.

The Quantitative Response of the Smallest Blood Vessels of the Human Skin to Graded Mechanical Stimulation and to Local Ischemia in Arterial Hypertension, Arteriosclerosis and Certain Allied Disorders. By JOSEPH R. DiPALMA and FRANCES I. FOSTER (introduced by J. Hamilton Crawford), Brooklyn, N. Y.

It has been repeatedly confirmed that the smallest blood vessels (precapillary arterioles, capillaries, and venules)

* From the J. H. Pratt Diagnostic Hospital and the Blood Clinic, Boston Dispensary.

take little or no part in the peripheral resistance of the hypertensive states. This, however, does not constitute absolute proof that such small vessels may not be involved in generalized vascular disease in ways other than can be demonstrated by the mere mensuration of arteriolar and capillary pressures. In this investigation, an attempt was made to elucidate this problem by other means.

The functional responses of the smallest vessels of the human skin were quantitated in suitable groups of hypertensive and arteriosclerotic patients, utilizing two methods recently devised and for which physiological, individual, seasonal, segmental, and aging characteristics have been described. One method measured the constricting responses of the small dermal vessels to graded mechanical stimulation; the other measured their ability to respond by reactive hyperemia to a standardized period of local ischemia. Comparison was made with normal data simultaneously obtained.

It was found that neither the patients with arterial hypertension nor those with arteriosclerosis had small dermal vessel responses in any way significantly different from the expected normal. There was no correlation between the severity or duration of the lesions and the quantitated responses. However, patients with the malignant syndrome of hypertension had responses which indicated small cutaneous vessels far less sensitive than normal. On the other hand, a number of hypertensive patients with various associated diseases, especially central nerve lesions, had small dermal vessels much more sensitive than the normal.

Avoidance of the Vasoconstrictor Action of Shed Blood in Perfusion of the Rabbit's Ear. By J. L. GUERRANT (by invitation), J. E. WOOD, JR. and E. M. LANDIS, Charlottesville, Va.

Attempts to perfuse the rabbit's ear with defibrinated blood from rabbits or dogs, normal or nephrectomized by the method of Page (J. Exper. Med., 1940, 72, 301) proved difficult in our hands because of immediate, or steadily increasing, intense vasoconstriction, beginning as soon as the diluted defibrinated blood entered the ear. In control observations, to maintain slow flow of defibrinated blood it was necessary to raise the perfusing pressure progressively. Assays of the pressor activity of heparinized plasma from hypertensive rabbits, dogs, and man were not convincing, partly because the tone of the auricular vessels increased unevenly in response to the perfusing fluid itself.

The intense constrictor action of heparinized whole blood and defibrinated blood on surviving blood vessels has been known for a long time and has been ascribed to powerful vasoconstrictor substances released by disintegrating platelets in latent or obvious clotting (Bayliss and Ogden, J. Physiol., 1933, 77, 34P, Janeway *et al.*, Arch. Int. Med., 1918, 21, 566). In agreement with these observations it was found that blood drawn from the heart of a normal rabbit into a syringe containing heparin and then perfused through the ear also produced

progressive constriction, though at a much slower rate than defibrinated blood.

It was observed, however, that the vessels of the rabbit's ear could be kept from constricting and could be perfused for two hours or longer, at a constant rate at a low and constant perfusion pressure, without edema, only if greatest pains were taken to avoid even slight latent coagulation. It was necessary to (a) heparinize the donor rabbit fully, (b) remove the blood into a syringe containing heparin, (c) dilute with heparinized Ringer Locke solution, and (d) bubble oxygen and CO₂ through the blood constantly. Cleaning all glassware of traces of constrictor material by preliminary soaking in alcohol or by boiling in sodium bicarbonate solution is also advisable. Under these conditions, the vascular tone remains low and variations in tone over a two hour period are small. The effect of vasoconstrictor substances can be demonstrated more clearly because these precautions eliminate the more vigorous constrictor action of substances associated with clotting.

In such preparations, it was found (a) that 0.2 cc. of defibrinated rabbit's blood always produces complete and persisting constriction, (b) that rabbit or human serum is only slightly less constrictor, (c) that heparinized plasma of normal rabbit or man is usually inactive or very slightly constrictor, and (d) that pressor substances of renal origin are far weaker than the vasoconstrictor substance of partially heparinized blood, serum, or defibrinated blood. It appears that assay of renin or allied substances in the blood of hypertensive animals or patients must be carried out with utmost care to avoid artifacts due to liberation of powerful constrictor substances in the course of latent or obvious coagulation, *in vitro* or even *in vivo*.

Analysis of Breathing Pattern. By JOHN L. CAUGHEY, JR., New York, N. Y.

Little clinical use is made of the thousands of spiographic tracings which accumulate in metabolism laboratories which employ the closed-circuit method for measuring oxygen consumption. To determine whether information of value could be derived from study of these spiograms, a method was devised for carrying out detailed analysis of breathing pattern.

Breathing characteristics which could be studied on the usual type of tracing, and which represented basic components of breathing were selected. These were rate, variation in rate, depth, variation in depth, volume of ventilation, expiratory pause, expiratory mid-position, sighs, swallows, contours of single breaths, and general regularity of the whole pattern.

Careful appraisal of these characteristics was made for a series of routine metabolism spiograms obtained from 500 female and 200 male patients who had a total of 984 tests.

On the basis of this material, a range of average breathing behavior under the conditions of the test could be defined. The results obtained suggest that: (1) breathing behavior may indicate poor attainment of "basal

conditions" during the test, (2) some types of breathing pattern are not compatible with accurate measurement of oxygen consumption, and (3) analysis of spiograms may give significant information about the physiological characteristics of the individual patient.

A Simple Method for the Laboratory Diagnosis of Sub-clinical Deficiencies of Thiamin, Riboflavin and Nicotinic Acid. By VICTOR A. NAJJAR (introduced by L. Emmett Holt, Jr.), Baltimore, Md.

The problem of assaying early deficiencies of these B factors has been approached by studying the urinary excretion of thiamin, of riboflavin, and of the fluorescent substance F_2 , the excretion of which has been shown* to depend upon available nicotinic acid. In previous work, by ourselves and others, several procedures have been employed:

- (1) The 24-hour urinary excretion,
- (2) Oral load tests,
- (3) Parenteral load tests, in which an arbitrary dose of vitamins is given subcutaneously, intramuscularly, or intravenously, and the urinary excretion is followed during the succeeding 3 or 4 hours.

Each of these procedures has been found to have serious objections. The 24-hour excretion tends to be low or absent in vitamin deficiency, but it is unfortunately influenced by the immediate vitamin intake; even markedly deficient individuals will excrete considerable amounts after ingesting a single vitamin-rich meal. When subjected to load tests, deficient individuals tend to retain the administered vitamin and non-deficient ones to excrete it. Oral load tests are, however, subject to errors caused by differences in rate of absorption, particularly in gastrointestinal disorders. The parenteral load tests avoid this particular difficulty but are open to two other objections: (a) The quantity of vitamin presented for renal excretion is so great that it may exceed the renal threshold, particularly if there is renal impairment, and the tests are therefore of limited value in subjects with renal disease. (b) We have found that the administration of one vitamin may influence the excretion of other B factors, and conversely, a deficiency of one B factor may influence the excretion of another B factor. Such vitamin interrelationships, which complicate the interpretation of excretion tests, are more conspicuous under "load" conditions.

In order to avoid the difficulties mentioned, we have employed a new procedure, designated as the *fasting "hour excretion" test*. We have found that the effects of ingested vitamin on vitamin excretion are transitory, being limited to the first few hours. Within 12 hours after a vitamin rich meal, the resulting surplus vitamin excretion has ceased and the excretion assumes a constant level which is dependent upon the body reserve of that particular vitamin.

* Najjar, V. A., Stein, H. J., Holt, L. E., Jr., and Kabler, C. V.: J. Clin. Invest., 1942, 21, 263.

The *fasting "hour excretion" test* is conveniently performed as follows. The subject is allowed to eat his ordinary evening meal. The following morning, 12 hours later, he voids on rising and discards the voiding. One hour after this he voids the specimen used for analysis, after which he is allowed to eat his breakfast. The specimen is analyzed for thiamin, riboflavin, and F_2 , the levels of these factors indicating the body stores of thiamin, riboflavin and nicotinic acid, respectively.

When the *fasting "hour excretion"* of any of these factors falls to zero it may be presumed that, for some brief period at least, there is a potential vitamin deficit, whereas a positive urinary excretion at this most critical period of the day (e.g. the longest period between meals) indicates that a surplus is available for urinary excretion and that the development of deficiency is not to be feared.

The *fasting "hour excretion" test* promises to give a more reliable answer than has hitherto been obtainable to the question of who needs vitamin supplements and who does not. We have studied it only in relation to the three vitamins mentioned, but it is possible that it may be applicable to other water soluble factors as well.

Sternal Marrow in Aplastic Anemia. By L. H. BEIZER (by invitation) and C. H. WATKINS, Rochester, Minn.

Sternal aspirations were carried out, and the bone marrow was examined, in 12 cases of refractory anemia in which the clinical picture was typical of aplastic anemia. Those cases presenting evidence of depression of the activity of the bone marrow, and having a relative lymphocytosis, followed the usual clinical course and death occurred within a short period of time. Those cases in which there was evidence of hyperplasia of the bone marrow, and in which a lymphocytosis was not observed, did not terminate fatally.

The Relation of Blood Pressure to Alpha Waves in the Tips of the Fingers Recorded Simultaneously with a Hamilton Manometer and a Plethysmographic Method. By CHARLES NEUMANN (by invitation) and ALFRED E. COHN, New York, N. Y.

This report deals with exploring whether alpha waves, as detected in finger tips, depend upon fluctuations in blood pressure. Simultaneous alpha waves and ipsilateral intra-radial blood pressures were recorded in ten adults, using a plethysmograph and a Hamilton manometer.

There were apparent a number of well recognized waves. All subjects exhibited slight inspiratory lowering of blood pressure which was concomitant with a small decrease in the volume of the finger tip (respiratory waves). Four subjects exhibited spontaneous fluctuations (10 mm. Hg) in blood pressure, occurring five times a minute (? Traube-Hering waves). These were accompanied by small (5 c. mm.) concordant fluctuations in volume of the finger tip.

But in addition there were many large (10 to 50 c. mm.) fluctuations (alpha waves) unrelated in time or direction to variations in blood pressure. From these data and from previous observations that the alpha waves

of adjacent fingers can vary independently, it is concluded (1) that the major rhythmic fluctuations in the volume of a finger tip have their origin elsewhere than in systemic blood pressure changes, probably in variations in local vasomotor tone, and (2) that rhythmic fluctuations in systemic blood pressure are accompanied by small concordant changes in the volume of the finger tips; that is to say, there are waves both dependent and independent of intra-arterial pressure.

During the past year and a half, it has been shown in this laboratory that the blood vessels of fingers, toes, and ears continuously undergo fluctuations in volume. As recorded by a sensitive pneumoplethysmograph these can be divided into five types of waves. Two are dependent upon the cardiac pulse and respiration. The other three are slower, larger and more irregular. Because they have not been shown to be related to other bodily functions, they have been named alpha, beta, and gamma. The alpha waves occur on an average of eight times a minute, with an average volume of 15 c. mm. (in a finger tip of 5000 c. mm.). Their rhythm is totally irregular, even for any one individual. The beta and gamma waves represent slower and larger variations in volume. There is evidence to show that in excitable individuals alpha waves tend to be large and variable in size, while in more placid individuals they are all small. Subjects have been classified into psychological types on the basis of their alpha waves.

*Neutralizing Antibodies to the Lansing Strain During the Acute and Convalescent Stages of Poliomyelitis.**

By THOMAS B. TURNER and (by invitation) LAWRENCE E. YOUNG, Baltimore, Md.

Numerous reports in recent years have indicated that a majority of normal adults have in their blood serum neutralizing antibodies against various strains of poliomyelitis virus, including the Lansing strain which has been adapted to mice. However, the relationship of the presence of these antibodies to onset or recovery in clinical poliomyelitis has not been clearly defined.

During the summer and early fall of 1941, blood specimens were obtained on 64 poliomyelitis patients in Baltimore at the time of admission to the hospital, and convalescent specimens at intervals up to 6 months. Acute and convalescent sera were tested simultaneously against the Lansing strain of virus, using 12 mice for each serum. Seventeen or approximately 27 per cent of the admission specimens completely protected the test groups of mice, 11 or 17 per cent showed partial protection, and 36 or 56 per cent showed little or no protection. In all but 10 of the 64 patients, the series of convalescent sera showed exactly the same degree of protection as the admission sera, and extensive titrations failed to reveal even slight differences. The changes observed in the sera of 9 patients were slight and occurred in both directions. The admission and one-month specimens from one patient failed to neutralize the virus, but a sample taken 4 months

after the onset of the disease showed definite neutralization.

It is clear that no relationship was demonstrated between the presence or absence of neutralizing antibodies to the Lansing strain and the onset of clinical poliomyelitis, in the outbreak studied. Moreover, recovery from acute poliomyelitis was not accompanied by a rise in the titre of these neutralizing antibodies. The significance of these findings were briefly discussed.

Effect of Addition of Dextrose Without Extra Insulin to Usual Regimen in Diabetics and Normals Upon Oxidation of Dextrose, Protein and Fat During Different Periods of Day. By JAMES A. GREENE and (by invitation) ANN DAVID, Iowa City, Ia.

Previous data indicated that a diabetic stored most of the dextrose which was added to the usual regimen without extra insulin, but clinical observations do not substantiate it. The dextrose, protein, and fat oxidized and caloric output have been ascertained, therefore, in diabetics and normals with usual diabetic regimen and with added dextrose without extra insulin, for 24 hours which has been divided into morning, afternoon, evening, and night periods. Normals oxidized over 90 per cent of added dextrose, whereas, diabetics oxidized only 50 to 60 per cent. Proportionately more was stored by diabetics, but urine excretion increased appreciably in 2 instances. Data for morning confirm previous observations indicating storage of most of the added dextrose, and as a rule less of it was oxidized during this period than during afternoon and evening. The oxidation per hour, however, does not show any consistent difference for the 3 periods. That for the night was consistently lower. The grams oxidized per hour were not related to height of blood sugar levels. Caloric output and oxidation of protein were not materially altered, but fat oxidation varied inversely to that of dextrose.

"Body Water" in Pneumonia. By DAVID D. RUTSTEIN, K. JEFFERSON THOMSON, DANIEL M. TOLMACH, ROBERT J. FLOODY, and WILLIAM H. WALKER (introduced by Walter S. McClellan), Albany, N. Y.

As a part of a study on the circulation of patients with typed pneumococcus pneumonia, the relationships of those simultaneous observations, indicated below, concerning "body water" were studied, and the results obtained during and after recovery from pneumonia were compared.

The following results were obtained: The plasma volume, total blood volume, and extracellular fluid volume were increased during pneumonia and returned to "normal" following recovery; the hemoglobin, red blood count, and hematocrit (mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration) were unchanged; and the plasma specific gravity and total blood chlorides were decreased during pneumonia and returned to "normal" following recovery.

The results of each of the above determinations were analyzed from the point of view of age, sex, pneumo-

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

coccus type, bacteremia, duration of disease, and extent of lung involvement. That analysis indicated that age is a significant factor in that young individuals tend to show these changes to a more marked degree than do older individuals.

The Relationship of Vitamin A to Infection in the Chick Embryo. By W. D. HAZELHURST, W. O. JOHNSTON, and E. W. PATTON (introduced by John B. Youmans), Nashville, Tenn.

The relation of vitamin A to infection has been studied, using the chick embryo as the test animal.

The diet of the pullets was controlled with respect to vitamin A as well as other foods. Eggs from the control and deficient group were incubated, and at the 13th to 16th day the embryos were inoculated with various strains of staphylococci.

Alterations in the vitamin A intake of the pullets resulted in parallel alterations in the vitamin A content of their eggs, and in the vitamin A content of the embryos developing from these eggs. The number of embryos surviving to the date of inoculation was significantly greater in the deficient group than in the control group.

A deficient supply of vitamin A did not influence significantly the course of infection in the developing embryo as judged by survival rates and blood cultures.

Infection of a chick embryo definitely reduced the vitamin A content of the liver and of the total embryo.

Separation of the Renal Anti-Pressor Substance from Certain Non-Specific Depressor Substances Present in Renal Extract. By T. R. HARRISON, J. R. WILLIAMS, JR., and (by invitation) ARTHUR GROLLMAN, Winston-Salem, N. C.

Since our report before this Society three years ago, describing the renal anti-pressor substance, efforts have been made to purify this compound. A number of different fractions which may lower the blood pressure of rats have been obtained:

(1) Histamine and similar toxic substances which are not precipitated by ammonium sulphate and are inactive orally.

(2) Non-dialyzable substances which are ineffective orally but may lower pressure when injected.

(3) Ammonium sulphate: This salt (which is used to precipitate the active principle) may, when fed in sufficient quantity, lower the blood pressure. It may be removed by extraction with aqueous picric acid.

(4) Certain as yet unidentified substances, possibly of phenolic nature.

(5) The renal anti-pressor substance which is present in the original ammonium sulphate precipitate and which passes through a dialyzing membrane may be precipitated from the dialysate by saturated aqueous picric acid. This is active orally and has little or no toxic effect. The amounts of this fraction needed in order to induce a striking decline in blood pressure correspond to large amounts of kidney.

The Effect of Changes in Body Temperature on Peripheral Circulatory Failure in the Mouse. By JOHN R. WILLIAMS, JR., Winston-Salem, N. C.

Peripheral circulatory failure, as seen in the terminal stages of infectious diseases, seems to differ from that seen in hemorrhage and trauma, the most outstanding variation being the presence of fever in the former. The effect of raising and lowering body temperature, and noting its effect on peripheral circulatory failure, has been studied in an attempt to further elucidate this phenomenon.

Peripheral circulatory failure was produced by the intraperitoneal injection of 50 per cent succrose in mice, a technique which is easily standardized.

Results indicate that mice kept at a body temperature of 39 to 40° C. appear more active and in better condition, but they die in a very short time. Mice kept at normal body temperature do not appear as well clinically but survive a considerably longer time. When the body temperature is lowered to 28 to 32° C., the mice become comatose and cyanotic and appear very ill. However, these mice survive longer and a higher percentage ultimately survive than in the other groups. The amount of fluid that exudes into the peritoneum in all three groups is approximately the same and is apparently not influenced by their body temperature.

*Observations on the Rate of Water Loss from the Skin of Humans in a Subtropical Climate.** By G. E. BURCH and W. A. SODEMAN, New Orleans, La.

The rate of water loss from localized surfaces of the body, including areas of high and low rates of clinical sweating, was studied quantitatively in a subtropical climate, in normal and diseased subjects. These areas included representative segments of the head, trunk, and upper and lower extremities. Observations were made under controlled atmospheric conditions which were varied from comfortable to hot environments, 75° F. and 50 per cent relative humidity and 95° F. and 75 per cent relative humidity respectively. They were taken in the winter and spring.

One hundred and ten observations were carried out on 31 subjects. In the normal subjects, a relative typical consistent pattern was found. The rate of water loss per unit time per unit area was greater in the finger tips and palm of the hands, toe tips and sole of the feet, and head, in that order, was lowest in the trunk, leg, thigh, and forearm, and intermediate in the axillae. A more or less uniform increase of water loss occurred in all areas following a change of the atmosphere of the room from a comfortable one to a hot one. Strikingly different findings were obtained in the diseased individuals. In scleroderma for example, impairment of water loss in the areas of high sweating resulted in a compensatory increase in areas normally low. A patient who experienced marked discomfort in a hot atmosphere failed to respond normally to increased environmental temperature. Similar observations of equal interest were found on other abnormal subjects.

*Financed by the Rockefeller Foundation, New York.

Quantitative Studies of the Effect of Concentrated Solutions of Human and Bovine Serum Albumin on Blood Volume after Acute Blood Loss in Man. By JAMES T. HEYL (by invitation), JOHN G. GIBSON, 2ND, ANNE SHWACHMAN (by invitation), LADISLAS WOJCIEK (by invitation), and CHARLES A. JANEWAY, Boston, Mass.

The preparation of highly purified albumin from human plasma by Edwin J. Cohn, Lawrence E. Strong, John L. Oncley, and S. Howard Armstrong, Jr., and the recent crystallization of bovine serum albumin by Edwin J. Cohn and Walter L. Hughes, Jr., has made available concentrated and stable solutions of these proteins as substitutes for plasma. It was felt important to investigate their action under carefully controlled conditions.

From 10 to 12 per cent of the circulating blood volume was rapidly removed from normal human subjects by venesection, and a 25 per cent solution of human or crystalline bovine albumin immediately given by vein. Changes in the blood volume were carefully followed over a three-day period, as measured by the values for hemoglobin, hematocrit, total protein, and plasma volume determined by the Evans blue dye technique. It was found that regardless of whether the subject was dehydrated or not, concentrated albumin solutions rapidly augmented the plasma volume. One hour after injection, the amount of fluid added to the circulation by each gram of albumin averaged approximately 17 cc. This is very close to the amount expected from calculations based on the related studies of the osmotic pressure of these albumin solutions by George Scatchard, Alan C. Batchelder, and Alexander Brown.

The physiologic response was identical with both types of albumin. If the preliminary period of dehydration was prolonged after the injection of albumin, a slight decrease in plasma volume occurred, which was not observed in subjects given salt and water by mouth. Serum and urine potassium did not increase following the administration of these hypertonic protein solutions.

Normal human serum albumin has been proven an osmotically powerful blood constituent, whose use in concentrated hypertonic solution has not been followed by untoward reactions. Although there have been no harmful effects from the injections after several months, further studies of sensitivity to crystallized bovine albumin must be made before its general use will be recommended.

A "Vitality" Stainable Structure in Young Erythrocytes. Its Relation to the Howell-Jolly Bodies or "Nuclear Rests." By SAVAS NITTS (introduced by Raphael Isaacs, Chicago), New York, N. Y.

When normal erythrocytes in early stages of development, from avian or mammalian embryos, or new born animals, or from bone marrow of adults, are placed in weak solutions of a "vital" dye, large, azure colored "droplets" develop slowly, long after the "reticulum" is formed. They vary in size, being single or multiple, and they are apparently not formed at the expense of the reticulum forming substance. When counterstained with

the Wright stain they resemble the nuclear chromatin in color, and are entirely indistinguishable from the so-called Howell-Jolly bodies, or "nuclear rests," observed especially in pathologic conditions.

When red blood cells from patients suffering from certain anemias, such as hemolytic icterus, erythroblastic anemia, sickle-cell anemia, hemolytic reactions caused by chemotherapeutic agents, etc., are placed for a few minutes in a weak solution of a "vital" dye, then filmed and counterstained, the percentage of cells showing "nuclear rests" is many times greater than that found on films prepared and stained in the usual manner.

It is presumed that the substance responsible for these formations is not of chromosomic or nuclear origin, but rather a lipoid, which fills the cell during its embryonic stage, and which enters into the formation of, or is replaced by, the hemoglobin, as the cell matures. It may be precipitated *in vivo* by a toxic agent, thus becoming readily stainable with the Wright, or similar stains, forming the so-called "nuclear rests." The nuclear rests stand in the same relation to the formations here described as "stippling" does to "reticulum," the former being precipitated *in vivo* by a toxic agent, the latter *in vitro* by a "vital" dye.

The Cause of Low Synovial Fluid Glucose Concentrations in Joint Disease. By MARIAN W. ROPES, ERIC G. L. BYWATERS (by invitation) and WALTER BAUER, Boston, Mass.

The present studies were undertaken to determine the cause of the low synovial fluid glucose concentrations that occur in some cases of joint disease. Significantly low concentrations are inadequate for the nutrition of articular cartilage, an avascular tissue that is nourished by synovial fluid. The normal fluid glucose level depends on the difference between the rates of entrance and utilization. In the past, such low concentrations have always been ascribed to increased utilization.

In order to test this assumption, we have studied the entrance of utilizable (glucose) and non-utilizable (thiocyanate) substances into joints with normal fasting synovial fluid glucose levels (traumatic type of joint disease) and joints with low fasting levels (rheumatoid and infectious arthritis).

In the former type, the appearance time of glucose was normal (20 minutes) and equilibrium was attained rapidly. Thiocyanate entrance was also normal. In the joints with low fasting fluid glucose levels, the appearance time of glucose was much slower (40 minutes) and the concentration rose only slightly in 2 hours. In such cases, thiocyanate entrance was slightly decreased.

To determine whether these findings were due to increased utilization or decreased entrance, similar studies were made with a non-utilizable sugar, galactose. Results very similar to those of the glucose experiments were obtained. The possibility of any utilization of galactose was excluded by intra-articular injection of galactose and demonstration of an essentially constant fluid level for 2 hours.

The results indicate that the rate of entrance of glucose plays a greater rôle than utilization in determining the fluid level. Normally glucose enters more slowly than thiocyanate. The rate of entrance is decreased in severe cases of rheumatoid and infectious arthritis even when thiocyanate enters fairly rapidly. Further evidence that the delayed entrance is not due to a decreased "permeability" is the fact that the entrance of proteins into the same joints is increased. The mechanism whereby the entrance of glucose is retarded is unknown. It seems probable that it is due to an intermediary chemical reaction which occurs fairly rapidly in normal synovial tissues but more slowly in certain types of joint disease. Elucidation of the mechanism will presumably indicate the mode of transfer of glucose to tissue fluid in all parts of the body, a process which even normally causes some delay in the entrance of glucose, as is most apparent in the case of spinal fluid.

Capillary Permeability as Determined by the Protein Content of Edema Fluid. Studies in Normal Subjects with Venous Congestion and in Patients with Acute Nephritis. By JAMES V. WARREN (introduced by Marshall N. Fulton), Boston, Mass.

Knowledge of the protein content of the capillary filtrate in a given portion of the body and of the extent to which physiologic or disease processes alter this fluid by changes in the capillary permeability is of both theoretical and practical importance. The purpose of this investigation was to determine the degree of capillary permeability during low-grade venous congestion and in acute nephritis.

Minimal pitting edema was produced in 8 normal subjects by obstructing the venous return from the leg for a 12-hour period. Thirty mm. Hg was chosen as the congesting pressure because this degree of venous obstruction produced little decrease in the resting blood flow of the part. From 1.5 to 25 mgm. of edema fluid were obtained by inserting several small needles into the subcutaneous tissue. The fluid was collected in capillary tubes of known weight and its nitrogen content was determined by the method of Kjeldahl. The average protein concentration of the edema fluid was 0.9 gram per cent. As the protein concentration of normal capillary filtrate is not known, it is impossible to say to what degree the increased venous pressure changed the capillary permeability. In view of these experiments, it is unlikely that the normal capillary filtrate in the leg exceeds 0.9 gram per cent protein; it is probably considerably lower.

The protein content of the edema fluid in 5 cases of acute nephritis ranged from 0.2 to 1.0 gram per cent, with an average of 0.5 gram per cent. The protein concentration was not significantly greater than that in cardiac edema. These data indicate that the edema of acute nephritis does not result from a generalized increase in capillary permeability, but from retention of salt and water because of renal disease.

The Comparative Effectiveness of Arsenical Compounds and Sulfonamide Drugs Against Bacterial Infections. By EDWIN E. OSGOOD, with the technical assistance of Inez E. Brownlee, Jane M. Armentrout, and Julia Joski, Portland, Ore.

The comparative effectiveness of neoarsphenamine, and many other trivalent and pentavalent arsenical compounds, and the sulfonamides, against many different bacteria was tested, using the technic of human marrow culture which permits controlled quantitative studies of the relative toxicity and effectiveness of drugs in an environment similar to that in the human body.

Arsenic trioxide and the six pentavalent arsenicals tested were relatively ineffective against all the organisms investigated. The ten trivalent organic arsenicals tested were more effective than any of the ten sulfonamides against all strains of staphylococci and most strains of *Streptococcus hemolyticus* and *Streptococcus viridans*. They were highly effective against diphtheria bacilli, gonococci, and *H. influenzae*, but were not as effective against these organisms as the most effective sulfonamides. The trivalent organic arsenicals were ineffective against pneumococci and of relatively little value against typhoid and colon bacilli.

In the concentrations safely attainable clinically, the order of decreasing effectiveness was neoarsphenamine, arsphenamine, sulfarsphenamine, trisodarsen, clorarsen, and mapharsen; for the sulfonamides, it was sulfathiazole, sulfadiazine, sulfapyridine, and sulfanilamide.

Neoarsphenamine, therefore, should be the drug of choice in many serious bacterial infections, and, of the sulfonamides, sulfathiazole and sulfadiazine should displace sulfanilamide as the drugs of choice in most bacterial infections.

The Absence of Erythrocyte Reserves in Human Subjects as Indicated with Radioactive Tagged Cells. By JOSEPH F. ROSS and MILAN A. CHAPIN (introduced by James M. Faulkner), Boston, Mass.

It is generally believed that the spleen is an important reservoir for erythrocytes, and that in times of stress (e.g. after hemorrhage or during shock) it contracts and liberates 20 to 30 per cent of the total erythrocyte volume into the vascular system.

Erythrocytes containing radioactive iron in their constituent hemoglobin are "labelled" during their lifetime, and can be detected quantitatively after injection into compatible human subjects. The total volume of cells in such subjects can be determined by applying the formula:

$$\text{Total volume of cells} = \frac{\text{Total radioactivity of injected cells}}{\text{Radioactivity per ml. of recipient's cells after mixing.}}$$

Volumes determined by this technique 10 minutes after injection of the tagged cells were similar to those observed 24 hours after injection. It is believed that 10 minutes is too short a period of time to allow mixing of the tagged cells with any considerable volume of im-

mobilized cells, although complete mixing would have occurred after 24 hours. The volume of cells in active circulation thus appears to be the same as the total volume of cells in the vascular system (including the splenic sinusoids) and no significant reserves of erythrocytes could be demonstrated.

The total volume of erythrocytes was not increased by administering adrenalin, although the venous hematocrit and plasma protein concentration increased 4 to 6 per cent. The increase in hematocrit was therefore assumed to be due to hemoconcentration or to redistribution of circulating cells and plasma in the vascular system rather than to the addition of more cells from the spleen or other hypothesized reservoirs.

Evidence for an Immune Mechanism as a Cause of Resistance to Insulin. By FRANCIS C. LOWELL (introduced by Chester S. Keefer), Boston, Mass.

Insulin tolerance tests with commercial crystalline insulin made from beef and pork pancreas and a preparation of human insulin were done in a diabetic patient exhibiting marked sensitivity and resistance to crystalline insulin.

Commercial crystalline insulin failed to produce a fall in the blood sugar, whereas human insulin caused a marked drop. Both types of insulin were capable of causing generalized urticaria. Passive transfer skin-sensitizing antibodies for both crystalline and human insulin were present in the patient's serum. The patient's serum was capable of protecting mice from convulsive doses of crystalline insulin but failed to protect mice against similar doses of human insulin. When the allergic antibody was destroyed by heating to 57° C., the insulin-neutralizing effect of the patient's serum in mice was still demonstrable.

These studies suggest that this patient had two immunological mechanisms involving insulin, one accounting for the allergic reactions, the other accounting for the resistance. Furthermore, evidence is presented that a patient resistant to insulin derived from beef and pork pancreas is not necessarily resistant to all insulins.

The Production of Microspherocytosis of Red Cells and Hemolytic Anemia by the Injection of Rattlesnake (Crotalus Atrox) Venom. By FRANK H. BETHELL and (by invitation) KARL BLEYL, Ann Arbor, Mich.

The venom of *Crotalus atrox* (Texas diamond back rattlesnake) was administered in sublethal and lethal doses to 12 dogs by either intravenous or subcutaneous injection. Within 30 minutes, a fall in blood pressure occurred associated with an increase in the cell plasma volume ratio and the concentration of plasma proteins. Subsequently, the plasma proteins fell, usually to a value below the pre-administration level. The cell plasma volume ratio continued to increase and the hematocrit value attained a level up to 50 per cent higher than the reading before administration of venom. Although the animals presented the picture of surgical shock, the rise in the hematocrit level could not be explained entirely by

loss of fluid from the vascular system, since it was not paralleled by increases in the erythrocyte count and hemoglobin value. Within a few minutes after the intravenous injection of either large or relatively small amounts of venom, the erythrocytes showed evidence of swelling and change from biconcave to more nearly spherical shape. A decrease in the mean cell diameter and an increase in the mean thickness were observed. Greatly decreased resistance to hypotonic salt solution occurred. By repeated administration of doses of venom too small to produce evidence of vascular damage it has been possible to induce hemolytic anemia, accompanied by hyperbilirubinemia and reticulocytosis, without apparent intravascular destruction of the red cells. This experimental anemia is analogous to familial hemolytic icterus with respect to changes observed in the circulating red cells. These studies support the view that the spherical and "fragile" erythrocytes characteristic of the latter condition do not represent an inborn error in formation of erythrocytes but rather an alteration in shape and resistance which occurs after their release into the circulation.

Kidney Extracts: Chemical Properties and Therapeutic Effects in Hypertension. By OTTO SCHALES and JAMES V. WARREN (introduced by James P. O'Hare), Boston, Mass.

Kidney extracts similar to those of Page and his co-workers have been prepared and administered to hypertensive patients. The concentration of the final product was such that 1 ml. represented 75 to 100 grams of fresh hog kidney. The extracts contained 2.5 to 4.3 grams of nitrogen in 100 ml., 23 to 28 per cent of which were present in the form of pseudoglobulins, while the rest existed in the form of proteoses, soluble in trichloroacetic acid, but precipitated by ammonium sulfate and by picric acid. Ammonium sulfate and other dialyzable material was absent due to prolonged dialysis at 0° C. The renin content was about 180 to 250 rabbit units (method of Schales and Haynes) in 1 ml. The extracts showed diastatic activity, 100 ml. producing 0.5 to 1.1 grams of reducing sugar from an excess of starch in 30 minutes at 37° C. All extracts were rich in hypertensinase. In 2 hours, 1 ml. inactivated *in vitro* all the angiotonin that is formed by incubating 1 to 3 liters of beef plasma with renin under optimal conditions.

Seven patients with malignant and essential hypertension have been given daily intramuscular injections of 10 ml. of this renal extract. In 2 patients no decrease in arterial pressure occurred. In 5 patients the pressure decreased after 5 to 10 days of treatment. Local reactions, often accompanied by fever, were observed. Inactivation of hypertensinase by incubating, for 9 hours at 37° C., extracts brought to pH 3.70 did not change the depressor effect of the material.

The data suggest that the depressor effect is probably better correlated with the occurrence of non-specific reactions than with a specific depressor agent.

Quinidine Hydrochloride with Urea Intramuscularly in the Treatment of Acute Cardiac Arrhythmias. By JOSEPH E. F. RISEMAN, and (by invitation) ELLIOT L. SAGALL, MELVIN I. STURNICK, and CHARLES HORN, Boston, Mass.

The sudden onset of paroxysms of rapid heart action, either spontaneous or complicating other conditions such as acute myocardial infarction, may require emergency treatment. Such episodes may be accompanied by vomiting, shock, or other conditions, making oral therapy inadvisable. No practical injectable preparations of quinidine have been available, however.

The addition of antipyrine and urea to quinidine hydrochloride results in a clear, colorless solution. This is sufficiently soluble so that a 2 cc. ampule containing 0.3 grams of quinidine hydrochloride can be prepared. This solution has been found suitable for intramuscular injection and has been stable for 9 months up to the time of writing. The present communication deals with measurements of the speed and duration of action of this preparation and the results obtained with its use in the treatment of cardiac arrhythmias in man.

The duration of action of cinchona derivatives on the human heart was estimated by repeated measurements of the Q-T interval of the electrocardiogram after oral and intramuscular administration of quinidine and quinine. These studies showed that all cinchona preparations in therapeutic dosages exert their maximum effect $1\frac{1}{2}$ to 3 hours after administration. Intramuscular injection produced earlier and more decided effects than oral administration of an equivalent dose. As a result of these studies, it was evident that if conversion of an abnormal rhythm did not occur within 2 hours after the administration of a dose of quinidine, additional medication would be required.

Twenty-three instances of arrhythmias in 19 patients were treated by intramuscular injection of quinidine hydrochloride with urea. The optimum initial dose was 0.6 grams. This was repeated or increased after 2 hours, depending on the response. This method of treatment was successful in all patients except 3 with sinus tachycardia. No untoward systemic or local effects were observed.

Studies on the Persistence of Pneumococci in Patients with Pneumonia Treated with Sulfonamide Drugs. By ROBERT A. GOODWIN (by invitation) and MAXWELL FINLAND, Boston, Mass.

Serial studies of sputum were made in patients with pneumococcus pneumonia, during treatment with sulfathiazole or sulfadiazine. The approximate numbers of the original and other types of pneumococci were estimated and the presence of other organisms noted. In about one-half of the cases, pneumococci disappeared from the sputum or throat cultures within 48 hours after drug therapy was started, and in another 30 per cent they could not be found after 3 to 10 days. In the remaining cases, those who were followed for more than 10 days were shown to have chronic or persistent basilar infection of

the lungs. In occasional cases, new types appeared during the course of treatment and after the original type could no longer be found. Antibodies developed in these cases at the usual time. Morphological changes, similar to those previously described by Frisch, were observed in the pneumococci during the course of sulfonamide therapy.

The Treatment of Bronchiectasis by Means of Continuous Postural Drainage (A Preliminary Report). By CLAUDE E. FORKNER and (by invitation) ALPHONSE E. TIMPANELLI, New York, N. Y.

An ideal to be accomplished in the medical treatment of bronchiectasis would be (a) to drain the bronchiectatic cavities by some method to keep them clean and dry; (b) to prevent resoiling of lungs with infected material from the upper respiratory tract.

These objectives can be accomplished by constant drainage with elevation of the middle of the bed on a suitable rack. Six patients have been so treated with encouraging results—decrease in quantity of sputum, improvement in the character of the sputum, decrease in cough, cessation of hemoptysis, gain in body weight, lessening of physical signs of the disease, and betterment of the general health of the patients.

In conjunction with this study, a preliminary series of observations showed that, in thirty-five unselected cases of bronchiectasis, 86 per cent had an absent (twenty-one cases) or markedly reduced (nine cases) pharyngeal reflex. A control series of normal adults exhibited this phenomenon in only 15 per cent of cases. No definite conclusion has been drawn from this observation, but it may have an important bearing as a causative factor in bronchiectasis.

Heat Production in Muscular Disease. By A. T. MILHORAT and (by invitation) J. D. HARDY and A. FAIR, New York, N. Y.

The heat production of 150 subjects, including 50 normals and 100 patients with muscular disease, was determined. The muscular syndromes included dystrophy, wasting subsequent to disease of the nervous system, and various disturbances of muscular function. Investigations of the metabolism of creatinine and creatine furnished data for computing the total functioning muscular mass of the body and for estimating the reduction in muscular mass due to wasting. Several of the patients were studied in the calorimeter of the Russell Sage Institute of Pathology, which permitted observations on direct and indirect calorimetry and respiratory quotient. In patients who had not yet reached puberty, muscular wasting was without significant effect on heat production, even when the excretion of creatinine and other observations suggested a reduction of as much as 60 per cent of the total muscular mass. In the adult patients, muscular wasting often was associated with decrease in total heat production, but this change was only slight or moderate in comparison with the reduction in muscular mass and in excretion of creatinine. These observations were

practically uniform in all the various muscular disorders that were investigated, with the notable exception of myotonia atrophica in which the decrease in heat production sometimes was greater than were the changes in muscular mass and function. The findings in this condition are postulated as being related to changes in organs other than the muscles.

The Influence of Secondary Factors on Induced Leukemia. By FRANKLIN R. MILLER, Philadelphia, Pa.

Cellular proliferations which resemble human leukemia may be induced in the organs of guinea pigs by the injection of extracts of urine from patients with the disease.

It seems possible that various factors such as infection, injections of proteins, anaphylactic shock, and exposure to benzol may have an influence on the induced disease.

Small doses of avirulent cultures of staphylococcus albus caused the death in twenty-four hours of animals previously prepared by injections with the urinary extract.

These animals had myeloblastic bone marrows and two had reductions of white blood cell counts to 600 prior to death. Normal animals given ten times the dose of the same culture of staphylococcus albus showed no ill effects.

Horse serum when given simultaneously with the injection of urinary extract increased the cellular proliferations in the organs of guinea pigs. With this combination the lymphoid disease also has been induced in rabbits. Treated animals which were sensitized to protein have shown few signs of anaphylaxis when tested for this phenomenon.

The injection of benzol prior to and simultaneous with the injection of urinary extract has not increased the disease, but in some instances seems to have retarded the process.

The lack of resistance to infection and the lack of anaphylaxis may indicate lowered immunity in these animals. A sparing action of one substance for the other may be elicited when benzol and urinary extracts are used together.

The Effect of Paravertebral Sympathectomy on Circulatory Functions in Essential Hypertension. By WRIGHT ADAMS and IRENE SANDFORD (introduced by C. Philip Miller), Chicago, Ill.

Seven patients with essential hypertension have been studied before and at intervals after removal of the paravertebral sympathetic chains from the stellate ganglion to the eleventh dorsal level or below. The data are not sufficient to justify conclusions regarding the value of this procedure in the treatment of hypertension, but its effect on some physiologic functions is uniform and in certain respects unexpected.

The pulse rate and oxygen consumption were progressively diminished during the first six months after operation, with little tendency to change during the second six months. The arteriovenous oxygen difference in-

creased during the first six months and decreased during the second six months in most cases. The cardiac output per minute was usually progressively reduced for the first six months. None of these changes was regularly related to blood pressure changes. Marked postural reduction of blood pressure occurred after operation, with little or no tendency to recovery during the first year. Measured exercise caused a further fall of blood pressure with comparatively little acceleration of the pulse.

The progressive nature of some of these changes is contrary to the usual concept that interruption of nervous pathways causes abrupt changes in function with a tendency to recovery. None of these patients showed clinical evidence of reduction of cardiac reserve.

Prenatal Electrocardiography. By ARTHUR J. GEIGER and (by invitation) ALLAN V. N. GOODYER and WILLIS M. MONROE, New Haven, Conn.

A clinically practicable technique for recording the electrocardiogram of the human fetus *in utero* has been developed by using a single stage resistance-coupled amplifier of simple construction in conjunction with a conventional portable electrocardiograph. The amplified fetal heart potentials were successfully picked up by disc electrodes on the pregnant subject's abdomen as early as the fourth month of pregnancy. Positive results have been found in 80 per cent of the tracings obtained during the last six lunar months of pregnancy, and with more recent refinements in technique the results have been practically 100 per cent positive in the last three lunar months.

The technique permits the prompt differential diagnosis of pregnancy from other abdominal tumors, it is free from false positive results, and it is less time-consuming than biological tests for pregnancy. A positive result is certain proof of life of the fetus.

The clinical value of the procedure is illustrated by twelve instances in which the question of dead fetus was answered correctly in all but one. Twin pregnancy has been recorded electrocardiographically in each of three available instances, and a case of triplets has been recorded.

Observations on the Vascular Volume and Blood Pressure in Minute Vessels of Patients with Hypertension and Certain Other Conditions. By J. C. HORTENSTINE (introduced by E. M. Landis), Charlottesville, Va.

By means of the pressure plethysmograph (Landis and Gibbon, J. Clin. Invest., 1933, 12, 105), graded external pressures ranging from 5 to 140 mm. Hg were applied to the forearm. At each pressure "dynamic vascular volume" was measured (a) by decrease in the volume of the forearm when circulation was stopped by inflating to 300 mm. Hg a narrow cuff at the axilla, and (b) by increase in the volume of the forearm when circulation was resumed after 2 minutes' complete occlusion.

In normal subjects, characteristic pressure-volume curves were observed in which the greatest "dynamic vascular volumes" appeared at external pressures between 20 and 35 mm. Hg. The maximal "dynamic vas-

cular volumes" averaged 1.3 cc. per 100 cc. of forearm for (a) and 1.8 cc. per 100 cc. of forearm for (b). These values were the same in subjects during fasting and after meals. Known grades of venous congestion reduced the "dynamic vascular volume" and moved the left limb of the pressure-volume curve toward higher pressures, without affecting the right limb. Simple dehydration lowered "dynamic vascular volume" without changing pressure relationships. Anemia had no effect on either volume or pressure. Free aortic regurgitation tended to move the pressure-volume curve toward lower pressures. Vasoconstriction and decreased circulating blood volume produced by venous congestion of 3 extremities reduced "dynamic vascular volume," and moved the summit of the curve to slightly higher pressures.

In hypertension, vascular volume remained approximately normal, but the summit of the pressure-volume curve was extremely flat and extended to pressures over twice the normal magnitude. The observations indicate that in hypertension the "dynamic vascular volume" is still approximately normal, suggesting, as have studies by others on blood flow, that the vasoconstriction of hypertension differs from vasoconstriction of neurogenic origin.

An Evaluation of the Use of Dicoumarin (3,3'-Methylenebis-4-Hydroxycoumarin) as an Anticoagulant, and Its Effect on Certain Plasma Constituents. By CHARLES S. DAVIDSON and HARRIET MACDONALD (introduced by Maurice B. Strauss), Boston, Mass.

The use of dicoumarin as an anticoagulant which may replace heparin in clinical medicine has been advocated by several investigators.^{1, 2}

The chemical nature of the substance differs widely from that of heparin, as does its physiological action. These physiological differences of action of the two substances are presented.

As compared to heparin, dicoumarin develops its anticoagulant effect slowly, and the recovery of the patient from the effects of the drug is much slower than from heparin, the effect of which is usually transitory.

Transfusion of whole blood in at least one case was inadequate for the control of the prolonged coagulation time produced by dicoumarin.

The coagulation time of venous blood measured by glass tubes does not give an index of the early changes in the coagulability of the circulating blood. The present observations suggest that the employment of lusteroid³ tubes shows marked changes in the coagulability of the circulating blood.

Dicoumarin causes changes in the plasma that are at the moment not fully understood. There is no doubt

that dicoumarin increases the coagulation time of the circulating blood and lowers the prothrombin concentration. There are certain characteristics of dicoumarin, such as difficulty of control, and its chemical nature, which suggest that caution should be used in the acceptance of the material as a general anticoagulant.

The Effect of Separate Inoculation of Vaccine Virus and Immune Serum on the Protection Test. By ROBERT F. PARKER, Cleveland, Ohio, and (by invitation) ROBERT H. GREEN, New York, N. Y.

Protection against infection with vaccine virus has been shown to result when immune serum is inserted intradermally in rabbits, with the virus inoculated later. This passive immunity gradually disappears. The present experiments were designed to measure the rate at which the protection is lost by inoculating graded amounts of virus at various intervals after the administration of serum. It was found that with the serum used, the amount of protection which was afforded declined regularly with time, but some was still demonstrable 96 hours after administering serum. Other experiments were done with the administration of serum by intradermal infiltration at different times after graded amounts of virus had been inserted. Although it has been stated that protection can be secured by this method only if the serum is inserted almost at once, the results of these more exact measurements show that good protection is afforded with a serum-virus interval of 6 hours. Slight, although probably significant, protection was obtained when serum was administered as late as 48 hours after virus inoculation. This is well within the incubation period for a lesion which might result from the small inoculum of virus.

A Statistical Study of Certain Etiologic Factors in Rheumatoid Arthritis. By CHARLES L. SHORT, WALTER BAUER and (by invitation) NATHAN R. ABRAMS and PHILIP E. SARTWELL, Boston, Mass.

Results are presented from a statistical study of 293 unselected patients with rheumatoid arthritis and a similar number of controls of corresponding age and sex. Our object was not only to confirm or refute some prevailing conceptions of the natural history of the disease, but also to uncover etiologic implications, especially by comparison with other diseases of less obscure origin.

Of the patients studied, 64 per cent were females, but this ratio was reversed in the 39 cases with spinal involvement. Special localizations of other diseases show a similar change in sex ratio. Our findings for the age of onset were compared with census figures for the age distribution of the population at risk and the chi-square test applied. No significant departure was found in males, but in females a marked increase was discovered in the age group 50 to 54. This finding suggests the influence of the menopause but our studies show no close relationship between the cessation of menstruation and the onset of the disease.

A significantly increased familial incidence of both rheumatoid arthritis and rheumatic fever was found in

¹ Butt, H. R., Allen, E. V., and Bollman, J. L., Proc. Staff Meet., Mayo Clin., 1941, 16, 388.

² Bingham, J. B., Meyer, O. O., and Pohle, F. J., Am. J. M. Sc., 1941, 202, 563.

³ Obtainable in the form of centrifuge tubes from the International Equipment Company, Boston, Mass.

patients as compared with controls, but the evidence is not sufficient to establish an hereditary factor. No relationship could be shown between rheumatoid arthritis and diseases of known allergic origin on the basis of familial or personal incidence of allergic manifestations. The validity of fatigue and anorexia as prodromal symptoms was established by means of questioning both patients and controls. Our data suggest that these and other constitutional symptoms may mark the real onset and that so-called precipitating factors, including acute infections and strain, merely determine a more easily recognizable phase with articular localization of the morbid process.

Changes in Blood Histamine Following Burns, Surgical Operations and Hemorrhage. By PAUL G. WEIL (by invitation) and J. S. L. BROWNE, Montreal, Canada.

Previous studies by Rose and Browne showed that the changes in blood histamine after severe burns could be divided into three phases: (1) an immediate, inconstantly occurring rise, (2) a fall below the normal level, and (3) a subsequent rise to or above normal. The second phase was the most constant and was associated with the period of edema and hemoconcentration. Further studies in twelve cases of burns confirm these findings for severe burns. In the case of mild burns showing slight or no hemoconcentration, the fall of blood histamine was slight or not present at all. The early rise occurred inconstantly as before.

Rose and Browne also reported lowered blood histamine levels after surgical operations. In cases developing shock, the blood histamine fell to low levels; in those not developing shock, it fell slightly. Twelve further cases have been studied before, immediately after, and at daily intervals, for one week after surgical operations. The previous findings are confirmed.

In order to estimate the role of hemorrhage in these changes, a study on blood donors was made. After loss of 500 cc. of blood in ten healthy male donors there was a fall to an average level 40 per cent below the initial level at the end of the bleeding which occupied about fifteen minutes. At this time, an average fall of 7 per cent in hemoglobin had already occurred.

In order to determine whether the decrease in blood histamine represented destruction or transfer to extravascular spaces, the cerebrospinal fluid histamine content was determined in four cases undergoing brain operations. There was no shock in these cases. The blood histamine fell moderately and simultaneously histamine which was absent before operation appeared in the cerebrospinal fluid, rose to a maximum at a time when the blood histamine was at its lowest level and disappeared again as the blood histamine rose. In general, the results suggest that a fall of blood histamine occurs in conditions in which a transfer of fluids to extravascular spaces is occurring and a subsequent rise in blood histamine takes place when fluid is being transferred in the opposite direction. The possible significance of these findings is discussed.

Gold Metabolism Following the Administration of Calcium Aurothiomalate and Aurothioglucose in Oil to Patients with Rheumatoid Arthritis. By R. H. FREYBERG and (by invitation) W. D. BLOCK and G. S. WELLS, Ann Arbor, Mich.

Patients with active rheumatoid arthritis were injected intramuscularly with different amounts of calcium aurothiomalate (a relatively insoluble salt suspended in oil); other patients were treated similarly with aurothioglucose (a readily soluble salt prepared in an oily suspension). The gold content of plasma, synovial fluid, and saliva, was determined frequently, and the daily excretion of gold in urine and feces was measured for many weeks during and after treatment. The plasma gold concentrations and twenty-four-hourly urinary excretion of gold of many other patients were determined at intervals during and after treatment with these same salts.

The gold content of plasma of patients injected with calcium aurothiomalate was always distinctly and often markedly less than previously observed after the administration of water soluble salts of gold (gold sodium thiomalate and gold sodium thiosulfate), containing equivalent amounts of gold. Similarly the excretion of gold was considerably less than occurred following injections of soluble gold salts. Rapid increases in plasma concentration and urinary excretion of gold never occurred following injections of the calcium salt, a result sharply in contrast to results after injection of soluble gold salts.

The plasma concentration and excretion of gold following administration of an oily suspension of aurothioglucose was usually about 70 per cent as large as obtained after the injection of comparable amounts of gold contained in aqueous solutions of other gold salts. Greater variations occurred when the oily suspensions were employed.

The significance and implications of the results will be discussed in regard to possible therapeutic and toxic effects.

Effects of Cozymase Upon the Growth of Staphylococci and Antistaphylococcal Action of the Sulfonamide Compounds. By WESLEY W. SPINK and (by invitation) JEAN JERMSTA VIVINO and OLAF MICKELSEN, Minneapolis, Minn.

West and Coburn have stated that cozymase (coenzyme I) inhibited the bacteriostatic action of sulfapyridine for the staphylococcus, whereas nicotinic acid and thiamin chloride did not. Strauss, Dingle and Finland were unable to confirm this finding. They pointed out, that although nicotinic acid and thiamin chloride are essential growth factors for the staphylococcus, cozymase would act as a growth stimulus in place of both nicotinic acid and thiamin chloride. We are reporting the results of our observations concerning the inhibitory effect of cozymase upon the bacteriostatic action of the sulfonamides, and also offering an explanation for the confirmed observation that cozymase will stimulate growth of the

staphylococcus equally as well in the presumed absence of both nicotinic acid and thiamin chloride.

Utilizing two preparations of cozymase, it was found that the material contained small but adequate enough quantities of thiamin chloride to support growth of the staphylococcus in a synthetic medium. It is reasonably assumed that the organisms, in the presence of nicotinic acid and thiamin chloride, utilize the former compound as cozymase for cellular reproduction. When certain experimental conditions were fulfilled, cozymase, but not thiamin chloride and nicotinic acid, inhibited the bacteriostatic action of sulfanilamide and sulfapyridine against staphylococci.

Experience with the Heller and Heller Test for Follicle-Stimulating-Hormone in the Urine in Endocrinological Diagnosis. By HARRY F. KLINEFELTER, JR., and GRACE GRISWOLD (by invitation), and FULLER ALBRIGHT, Boston, Mass.

With impaired gonadal function in either sex, one finds an excess of follicle-stimulating-hormone (FSH) in the urine if the trouble lies primarily within the gonads themselves. Hence, tests for excess of FSH have been very useful in endocrine diagnosis. Furthermore, the assumption has been made that, if an individual had obvious gonadal insufficiency and did not have an excess of FSH in the urine, the cause of the gonadal insufficiency was in all likelihood a decrease in FSH production. The present studies were planned to throw further light on this assumption.

In order to test the urine for a subnormal amount of FSH, it was first necessary to separate the toxic products from the hormone. This has been accomplished by the use of the Heller and Heller procedure which dialyses off the toxic products. The authors have used this method with minor modifications.

The present studies include observations on normal individuals to show how many mouse units of FSH can be expected in the urine of a normal individual per 24 hours. The remainder of the data concern quantitations of FSH in the urine of patients in whom there was reason to believe that the FSH was decreased or absent.

Antithromboplastin in Hemophilia. Effect of Intravenous Injection of the Hemophiliac's own "Thromboplastinized" Plasma. By LEANDRO M. TOCANTINS, Philadelphia, Pa.

Incubation of normal or hemophilic plasma, collected with especial precautions, with dilute aqueous extracts of brain tissue reduces the thromboplastic activity of these extracts. The antithromboplastic potency of hemophilic plasma is greater than that of normal plasma. This excess constitutes perhaps the primary defect responsible for the slow coagulation of hemophilic blood and the low thromboplastin content of citrated hemophilic plasma.

Antithromboplastin may be neutralized *in vitro* by incubating citrated plasma, for a given time interval, with sterile aqueous extracts of brain tissue. A transitory diminution in the venous blood coagulation time of hemo-

philiacs follows the slow intravenous injection of their own thromboplastinized plasma. There were no unusual symptoms, or any changes in the blood prothrombin and fibrinogen, following injections of the plasma.

Aseptic Meningitis of Known and Unknown Etiology. By JOS. E. SMADEL, New York, N. Y.

Materials from 165 individuals with aseptic meningitis have been studied in an attempt to establish the etiology of each patient's disease. Twenty-five of the group had choriomeningitis; this was proved by isolating the virus or by demonstrating complement-fixing or neutralizing antibodies in their convalescent bloods. Sera collected 2 to 5 weeks after onset contained the former antibody in 17 of 19 instances but the latter in only 3 of the 19. Subsequently, all 25 patients developed neutralizing substances and 23 had complement-fixing antibodies. Four years' experience with the complement-fixation technique has proved its value in the early diagnosis of choriomeningitis.

Five of 50 patients with aseptic meningitis not caused by the virus of lymphocytic choriomeningitis had in their early and late sera significant amounts of complement-fixing antibodies for psittacosis. The interpretation of these findings must rest on additional observations; however, the neurotropic tendencies of viruses of the psittacosis group in experimental animals are established and their capacity to produce aseptic meningitis should be considered, even though the present observations do not conclusively prove that this group of agents was active in these cases.

Six patients developed aseptic meningitis during or following mumps and were thought to have mumps meningitis. No etiology could be assigned for the disease of 129 of the 165 patients. Aseptic meningitis apparently is a clinical syndrome, caused by a number of infectious agents. The responsible agent should be identified, if possible, in each case.

The Fate of Colchicine in the Body. By AUSTIN M. BRUES, Boston, Mass.

The distribution and excretion of colchicine, following intravenous administration to rats, has been studied by means of a new colorimetric method, and the findings checked by bioassay using mice (for toxicity) and tissue cultures (for cytological action). Following injection, the blood concentration falls rapidly and reaches an almost constant level after a few minutes. This blood level is slightly higher than that in most tissues. Colchicine is excreted in bile, and also directly into the intestine, so that for several hours after administration between 10 and 25 per cent of the administered dose is found in the small intestine and its contents. This high intestinal concentration would appear to be responsible for certain toxic manifestations of the drug. There is no evidence for its specific accumulation in other tissues or in tumors. Urinary excretion occurs rapidly only during the brief period when the blood concentration is high. About 50 per cent of the injected dose has been recovered from whole mice

16 hours after injection. There is no evidence to support the belief that the delayed action of colchicine is due to its conversion to a more toxic compound; its cumulative action is probably dependent upon its prolonged retention in the body.

The Relation of a Somatic Factor to Virulence of Pneumococci. By COLIN MACLEOD and (by invitation) MACLYN MCCARTY, New York, N. Y.

The property of virulence of pneumococci has been generally assumed to depend almost solely upon the integrity of the capsule. That a somatic factor may also exert a pronounced effect on virulence is suggested by the occurrence of strains of encapsulated pneumococci which are entirely avirulent although culturally and immunologically identical with virulent strains of the same type.

In order to demonstrate more conclusively the relation of a somatic factor to virulence, the technique of type transformation has been used, whereby pneumococci of one type may be converted to another specific type by way of the rough intermediate.

Two strains of pneumococcus Type III, one virulent for the rabbit, the other avirulent, were transformed to pneumococcus Type II. The virulence of the newly-derived strains of Type II corresponded to that of the original Type III strains, indicating that the differences in virulence are related to a somatic factor and not dependent upon the capsule.

In other instances, the somatic factor may be present and yet masked because of the nature of the capsule. When a rough variant derived from a virulent strain of Type II pneumococcus was transformed into Types I, III, and XIV, the Types I and III were fully virulent and the Type XIV strain was avirulent.

The Effect of Sulfonamides on Virulence of Pneumococci. By FRANK L. HORSFALL, JR., New York, N. Y.

A quantitative study was made of the survival, growth, and virulence of pneumococci in solid media containing sodium sulfathiazole. The number of pneumococci and the concentration of sulfathiazole per unit volume of medium were varied independently. Only a very small but constant proportion of the total number of pneumococci were capable of growing in the presence of sulfathiazole and this proportion was inversely related to the concentration of drug. Secondary cultures derived from individual colonies grown once in sulfathiazole contained much higher proportions of cells capable of growing in the presence of the drug and these proportions were directly related to the concentrations in which the first growths had occurred. This increased capacity remained constant through numerous subcultures in drug free media.

The virulence of the organisms thus derived was decreased, often very markedly, although in all instances the pneumococci remained fully encapsulated and type specific. The degree of reduction in virulence was not definitely correlated with the concentration of sulfathi-

azole in which growth occurred. The reduced virulence did not change through numerous subcultures in the absence of the drug.

These results suggest that there are marked differences in the metabolic potentialities of individual cells of pneumococci. They indicate also that the cellular factor associated with the property of virulence, in contrast to the capsular factor, is susceptible of striking alteration as a result of a single exposure to the action of sulfathiazole.

The Relative Antimalarial Effects of Atabrine, Certain Acridine and Quinoline Derivatives, Quinine and Sulfonamides on Experimental Infections with P-Lophurae-Ducks. By O. W. BARLOW (introduced by Theodore G. Klumpp), with the technical assistance of E. Homburger, Rensselaer, N. Y.

Following the intravenous inoculation of young Peking ducks with infected blood (*P. Lophurae* Strain 12A), groups of birds were medicated with one of a series of preparations by incorporation of the medicament in the dry poultry mash (Bieter, *et al.*, 1939). Studies of the effects of various drug percentages in the diets permitted the quantitative evaluation of acridine, quinoline and sulfonamide derivatives with standard controls, *i.e.*, atabrine, plasmochin, or sulfanilamide and quinine.

The comparative order of efficiency of the various compounds tested from high to low is as follows: plasmochin > atabrine > P.2 > P.3 > quinine > acranil > neoacranil. Certain of these compounds on the basis of their margins of safety and therapeutic effects appear promising and merit further careful study.

Sulfamethylthiazole, sulfathiazole, or sulfadiazine are therapeutically active under the conditions of test. Their value appears to be in the order named, although inferior to the poorest of the above acridine derivatives. Therapeutic effects of combinations of these compounds with atabrine were not significantly superior to atabrine alone. No clear-cut therapeutic effects were demonstrable with sulfapyridine, sulfanilamide, sulfaphenylthiazole, or other derivatives.

Variations in Citric Acid Excretion During the Menstrual Cycle. By EPHRAIM SHORR and (by invitation) ALICE R. BERNHEIM and H. TAUSKY, New York, N. Y.

Citric acid has long been recognized as a normal metabolic intermediary, but its significance remains obscure. From work on minced tissue, Krebs has assigned it an important rôle in the oxidative metabolism of carbohydrates. Small amounts are present in blood (2 to 3 mgm. per 100 cc.) and 400 to 1200 mgm. are excreted daily in urine. Alkalies increase its urinary excretion; but, except for trivial amounts, ingested citric acid is oxidized.

The present studies have brought out a relation between urinary citric acid excretion and the menstrual cycle in women. Vaginal smear studies were correlated with the chemical observations. Urinary citric acid is lowest during menstruation, increases significantly throughout the intermenstrual phase, and again falls to

menstrual levels usually just before the onset of bleeding. In some cycles, a sharp transitory midmenstrual fall in excretion has been observed at about the twelfth day. Citric acid excretion is also significantly increased in amenorrheic girls by administration of estrogenic hormone. Apparently estrogenic hormone is at least one factor related to increased citric acid excretion of the intermenstrual period. The mechanism underlying the cyclic variation in citric acid excretion during the menstrual cycle is now under investigation.

The Subcutaneous Administration of Sulfathiazole Sodium in Various Clinical Conditions. By J. J. A. LYONS, D. R. CLIMENKO (by invitation) and L. W. GORHAM, Albany, N. Y.

The clinical efficacy of sulfathiazole in the treatment of certain infectious diseases is well established. One of the principal difficulties in the clinical use of drugs of this type is the necessity of maintaining therapeutically effective concentrations in the body over relatively long time intervals. Occasions arise when the oral administration of the drug constitutes a serious practical difficulty in the management of the patient, or becomes an actual impossibility. Under such circumstances, the usual practice is to administer the drug in the form of its sodium salt by intravenous injection. A practical difficulty is associated with the intravenous administration of the drug: under these circumstances, a peak concentration is reached in the blood, soon after administration is completed, which rapidly falls off as the drug is excreted. Excessive concentrations are rapidly followed by therapeutically ineffective concentrations. Therapeutic effectiveness attainable under this form of administration is far from optimal. This method of administration is admirable for rapidly attaining a distribution of the drug in the body, but it cannot be depended upon as a sole route of administration in the treatment of an acute infection.

Finland and his coworkers using the water-soluble glucoside of sulfapyridine, showed that this addition compound might be administered intravenously or subcutaneously. Unfortunately, they also pointed out that the glucose derivative was relatively inert from a therapeutic standpoint. Flippin advocated the intramuscular administration of concentrated (33 per cent) solutions of sodium sulfapyridine.

It has been repeatedly pointed out (Powell and Chen, and Marshall) that the alkaline solutions of the sodium salts of drugs like sulfapyridine are extremely irritant and that the subcutaneous administration of such solutions could cause severe local reactions with tissue destruction. We have found this to be true when concentrations of the order of 10 per cent or higher are administered subcutaneously or intracutaneously to experimental animals. We have also found that such experimental animals tolerate the subcutaneous administration of 1 per cent aqueous solutions of the anhydrous sodium salt of sulfathiazole without showing any sign of tissue reaction, and

that only a transient hyperemia results from the subcutaneous administration of 2 per cent solutions.

Our clinical evidence, which agrees with the observation of Taplin and his coworkers, bears out these experimental findings. Up to the present time more than 300 cases on the Medical, Gynecological, and Surgical Services of the Albany Hospital have received up to 2000 cc. of an 0.5 per cent aqueous solution of sodium sulfathiazole per day, administered subcutaneously by hypodermoclysis, without showing any untoward local reactions. This group was made up of a large variety of clinical cases which should have received sulfathiazole therapy, but in whom oral administration was contraindicated. It included such cases as abdominal and pelvic peritonitis, severe traumatic infections, late stages of lobar pneumonia, postoperative infections after section of the alimentary canal, acute infectious diseases in infants, etc. Both sexes and all age groups from infants through to advanced senility were represented in the series.

Blood concentrations of the drug during this type of medication were somewhat lower than would be expected if the same quantity of the drug had been administered orally. This is probably due to the fact that large quantities of fluid are administered and the resultant diuresis tends to speed up the passage of the drug through the body. This observation is substantiated by the small proportion of acetylated drug which is present in the tissues under these circumstances. Following oral administration, from 10 to 20 per cent of the drug in the body is conjugated, while less than 5 per cent of the total amount is conjugated after subcutaneous administration.

The Interrelation of the Venous Pressure, Tissue Pressure, and Blood Flow Through the Veins. By HENRY W. RYDER, WILLIAM E. MOLLE (by invitation), and EUGENE B. FERRIS, JR., Cincinnati, O.

Factors which influence venous pressure have been studied in intact and isolated veins and in models. When the vein under study is collapsed either by elevating it with respect to the heart level or by increasing the tissue pressure about it, the pressure in it is independent of the central venous pressure (pressure at any point nearer the heart) and exactly reflects the tissue pressure. When the vein is in such a collapsed state, the tissue pressure about it also becomes a function of the volume flow of blood through the vein. It is shown that the essential factor which causes this interrelation of venous pressure, tissue pressure, and blood flow in collapsed veins is the property of the vein of freely transmitting pressure across its wall over a wide range of volume change.

These studies demonstrate that when the tissue pressure is high enough to collapse the vein at any point between the heart and the point of measurement, the peripheral venous pressure becomes a function of this tissue pressure and is independent of right auricular pressure. Contrariwise, when the vein is distended throughout its course, the peripheral venous pressure is then a function of right auricular pressure and is independent of the tissue pressure. Thus, the collapse factor in veins is of great sig-

nificance in the interpretation of peripheral venous pressure.

Circulatory Changes Resulting from Trauma After Sympathectomy and After Spinal Cord Transection.

NORMAN E. FREEMAN and (by invitation) M. L. CULLEN and A. E. SCHECTER, Philadelphia, Pa.

Trauma to the limbs of dogs anesthetized with ether resulted in a reduction of blood volume greater than could be accounted for by the measured local fluid loss. Reduced circulation was consistently observed and preceded the development of shock. After total sympathectomy, even though the circulation was well sustained, a comparable reduction in blood volume occurred. In dogs, after recovery from spinal cord transection, trauma produced a loss of blood volume, although the circulation was well maintained as indicated by an adequate peripheral circulation, cardiac output, and normal oxygen saturation of venous blood obtained from the right heart.

It is concluded that reduced circulation after trauma is of diagnostic and prognostic significance but that the process of shock, as indicated by a loss of blood volume, may be initiated in spite of a well-maintained circulation.

Neuropathic Joint Lesions in Diabetes Mellitus. By C.

CABELL BAILEY and HOWARD F. ROOT, Boston, Mass.

Painless destruction of the joints of the tarsus has been observed in fourteen diabetic patients at the New England Deaconess Hospital during the last few years. The lesion has been observed to progress and to result in a peculiar type of deformity of the tarsus, regardless of treatment. Careful study has eliminated syphilis and the Charcot joint, and syringomyelia. The condition has occurred in patients with diabetes of long duration, with inadequate diabetic control, at ages varying from 30 years

to 60 years. Certain of the patients have had associated diabetic neuritis. One patient has come to autopsy and a complete examination of the joints of the foot and of the central nervous system is reported. The condition is not dependent, apparently, upon deficient blood supply. As a result of the diminished sensation, infections of the toes and skin later occur and sometimes become chronic. The etiology is not yet determined but seems to be of neuropathic type.

Further Observations on the Esophageal Electrocardiogram. By JAN NYBOER (introduced by Herman O.

Mosenthal), New York, N. Y.

The esophageal electrocardiogram has frequently been employed in the study of arrhythmias, while Hamilton and Nyboer first emphasized its importance in the study of posterior myocardial infarction. These studies have been extended to include cases in which the standard leads show atypical QT patterns and in cases suspected of having multiple myocardial infarction. The observations clearly show that lesions of the anterior and posterior myocardial wall, respectively, may be demonstrated by multiple exploratory leads in the anterior and posterior ventricular regions.

In order to enlarge on the concept of the electrical field, typical cases of left ventricular hypertrophy, right ventricular hypertrophy, right bundle branch block, left bundle branch block, and digitalis effects have been obtained. Cases of bundle branch block, associated with or resulting from myocardial infarction, reveal patterns which are worthy of this esophageal, electrocardiographic study. On the basis of our limited experience, it appears that knowledge of the electrical field of the posterior heart region becomes definitely helpful in evaluating the normal and abnormal standard lead electrocardiogram.

A STUDY OF THE INFLUENCE OF THE CHARACTER OF AN EXAMINING ROOM ON THE PERIPHERAL BLOOD VESSELS OF NORMAL, HYPERTENSIVE, AND SENILE SUBJECTS¹

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The decor, the very furnishings of a room, have turned out to be decisive in the results of examinations of certain kinds of physiological behavior. Circulatory reactions which did not occur or occurred to a less or slight degree in a room equipped like a laboratory, were more extensive and more nearly uniform in a room furnished like an ordinary bedroom. The results of investigations intended to describe "basal" conditions, whether psychological or physiological in a more general sense, may all be subject to changes away from familiar environments. The fact that the environment in which such studies are undertaken influences the result is well known and has no doubt received attention both from physiologists, as from Pavlov in the case of animals, and from psychologists, both practical in the case of decorators, and academic in the schools. Although known, there is scarcely a reference to the bearing of this factor upon the results of such investigations as are now being reported.

These studies were concerned with quantitative measurements of spontaneous variations in the volumes of fingers and toes and of the time of reaction (constriction) of small blood vessels following the application of sensory stimuli at distant parts of the body (1, 2).

The apparatus consisted of a camera by means of which the movements of a sensitive membrane, activated by the pulsations of a finger tip, could be recorded. The only part of the apparatus in contact with the patient was a lightweight chamber which fitted loosely over the terminal portion of the tip of a finger or toe. Application of a resinous material was relied upon to seal the chamber in place. The tips of fingers and toes of all patients underwent rhythmic variations in volume, which were recorded as a series

of waves whose upward deflections signified increase in volume of the part, and downward deflections, a decrease. For the most part, such variations in volume occurred without any obvious cause. It was soon learned, however, that downward deflections could be occasioned by subjecting the patient to a sensory or ideational stimulus. The present report is an attempt to ascertain some of the conditions which allow such decreases in volume to take place, and is presented separately from a more detailed description of the purely mechanical aspects of obtaining the data in view of the unexpected new information which was uncovered, not by improvement in technique, but rather by closer control of the environmental aspects of the experiment.

Our experiments were first carried out in a medium-sized air-conditioned laboratory, furnished with a typical hospital bed, two or three small chairs, and a mass of unfamiliar equipment such as a large laboratory sink and a table on which were kept unused pieces of apparatus. The recording capsule, camera, and timers were screened from the patient by forbidding black, reasonably light-tight curtains, through which he could hear distinctly noises and clicks incident to the use of the apparatus. He was always aware of the presence of the observers, even though he could not see them. The apparatus for the application of stimuli (heat, cold, pin-prick, touch) was hidden also by a curtain. The observer was separated from the subject by still another curtain. At no time could he predict the moment of application of a stimulus. Person after person commented upon the fact that he felt himself under a state of considerable "tension" and apprehension. The darkened "laboratory", the type of room, the knowledge that persons were working immediately behind screens near his bed, and the anticipation of something unexpected contributed to the state of his feeling. Some patients disarmed

¹ This is the 7th paper reporting the results of studies of the small blood vessels and related subjects.

² Commonwealth Fund Fellow.

even under these circumstances but that was the exception.

Obviously, the most desirable time to carry out observations like these is when individuals regard themselves as being, and are, in a state of relaxation. Care has not usually been taken in such studies to see to it that these conditions are actually attained. Accordingly, certain changes were made in our examining room in the hope of putting subjects at greater ease. The laboratory was divided. One part remained laboratory; the other part became bedroom. A one-way mirror permitted the observation of patients from the laboratory. The bed and walls were draped with material of a pleasing design. The room was furnished with a small bed-table, a Morris chair, rugs, lamps, magazines, coat rack, and pictures. A subject entered, not a frightening laboratory equipped with strange machines, but was conducted into a comfortable room, not too dissimilar from his own. He came into contact not with a staff of observers but with one physician only who put plethysmographic cups in place and explained that they led to the rest of the apparatus located in a distant part of the building. The records were in fact made in the adjoining laboratory by an observer who could see and hear what was going on. Communication between the observers though separated only by a wall was carried on by telephone to create the illusion of distance. The stimuli were not applied until the patient had been left alone and was resting quietly. As stimulus, use was made of a telephone-like bell rather than of an unfamiliar noise,

and for light, a bright diffuse light. Both stimuli were set in operation from the adjoining room. When it came time to stimulate with heat, cold, or pin-pricks, the first observer reentered the bedroom and raised a small white curtain between the subject and himself. It would have been better to apply all stimuli mechanically but this method was adopted to avoid the need of affixing appliances to the subject's body.

In the original "laboratory", it was noticed that most of the normal subjects "relaxed" and rested comfortably while the patients with hypertension did not. It was found also in 15 normal subjects that 160 stimulations were followed by 117 (73 per cent) instances of vasoconstriction, while in 13 patients with essential hypertension, vasoconstriction was less frequent (102 of 224 stimuli or 42 per cent). In the 10 senile subjects, this reaction occurred in 46 of 107 stimulations (43 per cent) (Table I). The normal subjects were either technicians or were otherwise familiar with laboratories. This was not the case with the patients with hypertension and the senile subjects. It was noticed that if recordings were obtained without the subject's knowledge, he became more "relaxed" as evidenced by the increase in size of the pulse and alpha waves (1, 2), and developed vasoconstriction more readily following stimulation. In any event, everyone subjected to the tests frequently commented unfavorably upon the apparatus, curtains, darkness of the laboratory, small beams of light, and the presence and movements of the observers. Of them all, hypertensive patients were most offended.

TABLE I

The effects of environmental conditions upon the incidence of vasoconstriction in the blood vessels of the finger tips of normal and senile subjects and patients with hypertension following the application of sensory stimuli to another part of the body

Subjects	Number	Type of room	Number of responses of blood vessels to application of a sensory stimulus		Total stimuli	Per cent of stimuli followed by vasoconstriction
			Vasoconstriction	No response		
Normal	15	Laboratory	117	43	160	73
		Bedroom	44	19	63	70
Hypertensive	13	Laboratory	102	142	244	42
		Bedroom	22	12	34	65
Senile	10	Laboratory	46	61	107	43
		Bedroom	32	20	52	62

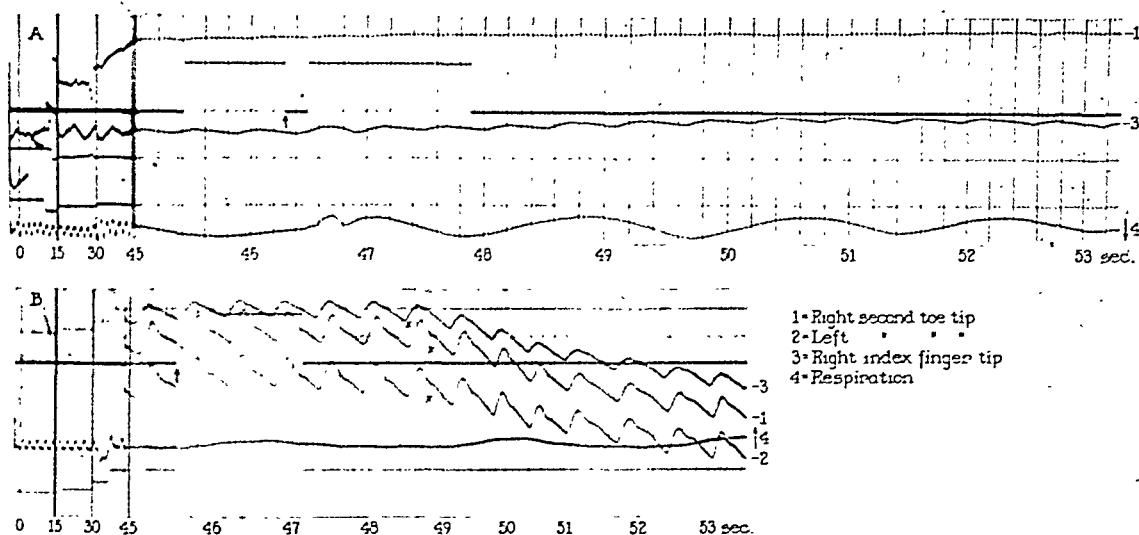


FIG. 1. PLETHYSMOGRAPHIC RECORDS OF THE TIPS OF THE FINGERS AND TOES OF A PATIENT WITH HYPERTENSION

Sensory stimuli were applied at the arrows. The pulse and alpha waves are on the same scale.

A. A representative record is shown taken during one of 11 separate observations on the subject in the "laboratory" type of environment. The blood vessels of the finger and toe tips are spastic as indicated by the small size of the pulse and alpha waves before the application of the stimulus.

B. A representative record is exhibited taken during the first observation in the bedroom. The vessels are much less constricted than in Figure 1A. The vasoconstriction began at the points marked by X, about 3.3 seconds after the application of the stimulus.

After the laboratory was changed and the new room put into use, the frequency of vasoconstriction following stimulation increased markedly in the patients with hypertension and the senile subjects, equalling the incidence in normal persons. In the senile subjects, vasoconstriction occurred in 32 of 52 (62 per cent) trials; in the patients with hypertension, in 22 of 54 (65 per cent); and in 44 of 63 (70 per cent) in normal individuals, that is to say, 73, 42, and 43 per cent as against 70, 65 and 62. Everybody avowed voluntarily that he found the new environment to be comfortable, conducive to relaxation and that he was much less apprehensive shortly after having been left alone.

The behavior of 2 patients with hypertension deserves separate comment. In the "laboratory," they remained "tense" and very apprehensive on 10 and 11 separate observations, respectively, spread over a period of 3 months. It was with great difficulty that a few reactions were obtained. But on the first trials in the new environmental conditions, they "relaxed"; practically every stimulus was followed by vasoconstriction (Figure 1).

COMMENT

The fact that relaxation occurred derives its confirmation from two circumstances. First, from the statements of subjects, practically all of whom asserted they felt more comfortable and under less tension in the bedroom than in the laboratory where they could not but feel acutely that they were being experimented upon. And second, from the appearance of the subjects and from the plethysmographic records themselves. In general, when the pulse waves were *small*, a stimulus would not bring about much, if any, vasoconstriction; but when *large*, except in a few senile subjects, the likelihood of vasoconstriction was greatly increased. It was very common for hypertensive subjects, in particular, to exhibit small pulse waves in the "laboratory" and large ones when the room was made pleasant. At times, patients would volunteer the information that they felt relaxed in the "laboratory". Without exception, examination would then reveal that the pulse waves were large.

Vasoconstriction consists of two factors, de-

crease in the size of the pulse waves and also in that of the alpha waves. It is a special type of the latter which documents the major decrease when the subject reacts to a sensory stimulus (2).

Even without the subjective and objective data which have been accumulated to support the idea that a familiar room is more agreeable than a strange one for these particular purposes, subject or observer alike would select the new rather than the old room, especially if the observer wished to avoid as many disturbing factors as possible.

There is another factor which may decide the choice of environment. The avoidance of pain or of a painful impression does not necessarily give a complete picture. A room arranged as a laboratory may place a certain desired strain upon individuals and by so doing, bring out differences which might otherwise have been overlooked.

Although the hypertensive and senile subjects showed a higher percentage of reactions in the bedroom than in the laboratory, no such difference was noticed in the normal subjects. But our "normal" subjects were, as noted above, acquainted with the hospital and were familiar even with the particular laboratory used. The changes in the room could hardly have made much difference to them. However, the frequency of reactions was raised in the hypertensive and senile subjects approximately to the level of the normal group by just a few simple changes in the environment. Had the old room continued to be in use, the conclusion would have been drawn that there was a difference in the three groups of subjects, but in

the new room, the conclusion is obvious that the same reaction is possible and took place in all groups.

SUMMARY

Objective evidence supports the belief that the conditions under which physiological studies are carried out must be suitably arranged, not only to assure uniform temperature, humidity, and state of digestion, but also less tangible factors such as the patient's mental comfort and the degree of his relaxation. This was demonstrated by converting a "laboratory" into a conventional bedroom and by observing how the frequency of reaction on the part of peripheral blood vessels increased when sensory stimuli were applied at distant parts of the body. This observation was made not only in the case of groups of hypertensive and senile subjects but also in individual subjects studied under both types of environment. Conversely, in tense individuals, to be unable to relax in the atmosphere of a "laboratory" is evidence of the possible presence of an abnormal process.

REFERENCES

1. Burch, G. E., Cohn, A. E., and Neumann, C., A study by quantitative methods of the spontaneous variations in volume of the finger tip, toe tip, and postero-superior portion of the pinna of resting normal white adults. *Am. J. Physiol.*, 1942, 136, 433.
2. Burch, G. E., Cohn, A. E., and Neumann, C., Reactivity of intact blood vessels of the fingers and toes to sensory stimuli in normal resting adults, in patients with hypertension, and in senile subjects. *J. Clin. Invest.*, 1942, 21, 655.

REACTIVITY OF INTACT BLOOD VESSELS OF THE FINGERS AND TOES TO SENSORY STIMULI IN NORMAL RESTING ADULTS, IN PATIENTS WITH HYPERTENSION, AND IN SENILE SUBJECTS¹

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That small blood vessels may react by constriction is well recognized. It is probable, in addition to smooth muscle, that there are other contractile elements. The sympathetic nervous system is generally regarded as playing a prominent role in their function. In certain states of disease and stress, the effect of this control is manifest in blanching, cyanosis, and coldness, or the reverse of these. Previous studies (1 to 3) have shown that small blood vessels undergo a continuous series of changes in size even in the resting subject. It is the object of this paper to describe the effect of applying sudden stimuli which disturb this resting state.

Although it would be possible, using figures for the chronaxie of the blood vessels in isolated strips of animal tissue (4 to 6), to estimate that of human blood vessels, this information, however valuable, is not necessarily applicable to the situation in intact human subjects. It became desirable, therefore, to adapt an older method, capable of yielding information which could be used to characterize the responses in man. Fano (quoted by Luciani (7)), many years ago, described the details of a method which made possible the measurement of changes in size of anatomical parts when various stimuli were applied at remote points. But Fano's apparatus was not sensitive to small changes. His results were disturbed, furthermore, by the inclusion of large arteries and veins in his plethysmographic chambers. In the present studies, the small blood vessels are of prime interest, making it desirable to choose the tips of fingers and toes. But even these parts contain arterioles, venules, capillaries, and arterio-venous communications, so that any result must be interpreted as representing the combined effect

of the activity of many structures. In addition, it must be remembered that the pathway from the point of reception of a stimulus to the blood vessels involves many interconnecting structures, including the receptors, sensory nerves, synapses, the pathways within the central and autonomic nervous system, and neuromuscular junctions. Measurements in intact subjects give information concerning the time taken by the blood vessels to react only when the time required to traverse these complex pathways is taken into account. In point of fact, it is only in the post-ganglionic fibers of the sympathetic system that an interval of importance relative to the time of reaction of blood vessels is to be expected.

It is unknown what role the reactivity of small blood vessels to sensory stimulation plays in arterial hypertension. The cold pressor test (8) was developed to distinguish between individuals whose blood vessels are unusually reactive and who are potentially hypertensive and those whose blood vessels react in a normal fashion. An effort has now been made to characterize further the blood vessels of hypertensive individuals by obtaining data, not only on the degree of reaction, but also covering the time of reaction. For contrast, a group of senile subjects has been studied.

METHOD

The apparatus employed in these observations was essentially that previously described (3). It was modified for special purposes, as will be described later. It records simultaneously the changes in volume of the tips of the right index finger and the right second toe with a sensitive pneumoplethysmograph (9). The subjects rested in bed in an air-conditioned room (temperature 75° F. \pm 1°; relative humidity less than 50 per cent) at least 30 minutes before recording was started. In an early period of this study, the subjects rested in a room containing the apparatus as well as other laboratory equipment. Later, because of psychic disturbances produced by this unfamiliar environment, the examining room was reded-

¹ This is the 9th paper reporting the results of studies of the small blood vessels and related subjects.

² Commonwealth Fund Fellow.

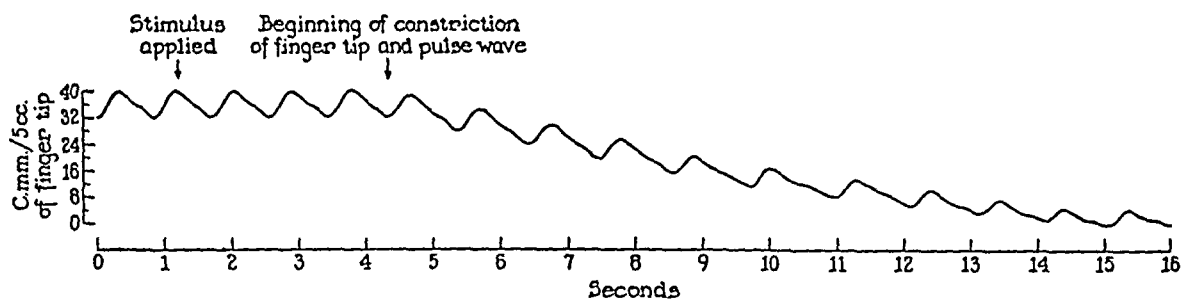


FIG. 1. A SCHEMATIC DRAWING SHOWING THE AVERAGE TYPE OF REACTION IN THE FINGER TIPS OF ALL SUBJECTS

The units of time and volume and the rate of vasoconstriction are the mean values obtained.

orated to resemble a bedroom (10). Its walls were made sound proof. After plethysmographic cups were sealed in place, the tips of the fingers and toes were adjusted to the level of the subject's heart. The physician then departed. Evidence that subjects were "relaxed" appeared in the increase in volume of the pulse and alpha waves (3).

Once a subject reached a state of maximum relaxation, stimuli were applied and the reactions of the blood vessels, evidenced by changes in volume, were recorded. When stimuli were applied in order to measure the reaction time, the speed of the photographic paper was about 4 to 6 cm. per second. Between stimuli, the paper ran at a rate of 8 cm. per minute. The time of application of each form of stimulus was registered on the photographic record by a light beam, activated by a magnet. In each case, the act of applying the stimulus closed a circuit of which the magnet was a part.

The stimuli employed were light, sound, heat, cold, pin-prick, and electric shock. Light, as a stimulus, was supplied for one second by a 500-watt photoflood lamp. Sound was made by ringing a telephone bell, 0.4 second, 3 feet from the subject. A sudden loud sound was produced by firing a cap-pistol about 3 feet away. Heat and cold were applied by touching the subject's arm on the external surface just above the left elbow with a metal plate (11 sq. cm. in area) at 50° C. and 3° C. respectively. Pain was produced by a pin-prick of uniform force on the external surface of the arm, just above the left elbow. Electric stimulation was supplied by discharging a condenser (4.5 microfarads) at the same point. Contact between the electrodes and the subject's skin was made with a sodium chloride jelly, the resistance being adjusted to about 5000 ohms.

Subjects were not warned that they were to be tested for their reaction to certain stimuli. In the case of light and the bell, they were left alone. In the use of other stimuli, to avoid complicated apparatus, it was necessary that an observer enter the room. He erected a curtain between the subject and himself. The subjects did not know the order in which stimuli would be selected nor the time of their application.

Subjects were regarded as being relaxed when, after a reasonable period, their pulse and alpha waves had attained maximum proportions. Response to stimuli was documented by decrease in size of these waves. Re-

covery was known to have taken place on return of the waves to their original volumes.

The reaction time to a stimulus was the time taken for a change in volume to begin (Figure 1). To study changes in volume, only the finger tips were measured. The measurements made were: (1) the initial volume of the pulse wave, (2) the volume of the pulse wave at its minimum, (3) the maximum change in "alpha" waves,³ (4) the time required for the "alpha" wave to reach a minimum, (5) the time required for the "alpha" wave to return to the original volume, and (6) the phase of the "alpha" wave when the stimulus was applied.

The reaction was identified as beginning at the intersection of a line joining the foot points of the last few pulse waves before the onset of vasoconstriction (decrease in size of the waves), with another joining those just afterward. The study of a pulsating model suggested that this point was located with an error no greater than 0.05 second.

The subjects chosen for investigation included 17 normal individuals (12 males and 5 females), varying in age from 11 to 43 years, 12 hypertensive patients (6 males and 6 females), varying in age from 14 to 54 years, and 11 male senile subjects, varying in age from 70 to 85 years. All of the subjects were free from renal or cardiac failure. Each subject was studied at least twice and some as often as 12 times.

RESULTS

The mean time of reaction of the finger tips of the normal subjects in 178 responses was 3.12 ± 0.02 seconds, with a standard deviation of 0.33 ± 0.01 second and a coefficient of variation of 10.6 ± 0.04 per cent, and that of the toes in only 63 responses of many trials, 3.42 ± 0.04 seconds with a standard deviation of 0.49 ± 0.03 second and a coefficient of variation of 14.4 ± 0.9 per cent. The means of the two (fingers and toes) were significantly different (3.12 as against 3.42 seconds). Although the toe usually reacted after

³ The reason for the use of quotation marks about the alpha will be explained later.

the finger, in rare instances the order was reversed. The mean reaction times for each stimulus in the finger tip were: bell, 3.33 seconds; light, 3.26; sound, 3.07; cold, 3.07; heat, 3.04; pin-prick, 2.95; and shock 2.86 (Table I).

The mean time of reaction of the vessels of the tips of the right index finger of *patients with hypertension*, in 159 responses, was 2.94 ± 0.03 seconds, with a standard deviation of 0.50 ± 0.02 second and a coefficient of variation of 17.0 ± 0.7 per cent; and of the tips of the right second toe, in 56 responses, the mean reaction time was 3.24 ± 0.04 seconds, with a standard deviation of 0.50 ± 0.03 second and a coefficient of variation of 15.4 ± 1.1 per cent (Table II). The means of the reaction times of the fingers and toes differed by 0.30 second. This was found to be statistically significant. The mean reaction times for each stimulus in the finger tips were: light, 3.39; sound, 3.00; bell, 2.92; heat, 2.91; cold, 2.89; and pin-prick, 2.81 seconds. These mean values cannot be considered significantly different because of the limited number of reactions to each stimulus. In

the toe tips, the mean reaction times for each kind of stimulus showed similar variations.

The mean reaction time in the tips of the right index finger of the *senile subjects*, in 80 responses, was 3.86 ± 0.04 seconds, with a standard deviation of 0.58 ± 0.03 second and a coefficient of variation of 14.9 ± 0.8 per cent; and in the tips of the right second toe, in 28 responses, it was 4.25 ± 0.07 seconds, with a standard deviation of 0.54 ± 0.03 second and a coefficient of variation of 12.7 ± 1.2 per cent (Table III). The mean reaction time in the toes was 0.39 second longer than that of the fingers, a difference which is statistically significant. The mean reaction times for each kind of stimulus in the finger were: heat, 4.16; light, 4.14; pistol shot, 4.06; pin-prick, 3.92; cold, 3.92; and bell, 3.58 seconds. The number of reaction times for each kind of stimulus was so small as to make it impossible to be certain that any of these differences was significant. The variations of the mean values for each kind of stimulus in the toes were of the same order (Table III).

The total number of satisfactory responses ob-

TABLE I

Mean values of various phases of the vascular response in the tips of the right index finger and right second toe in 17 normal resting subjects

Stimulus	Finger tips										Toe tips	
	Number of reactions to stimulus	Volume of pulse wave before stimulation	Volume of pulse wave when finger reached minimum volume	Decrease in volume of pulse wave	Maximum decrease in volume of part	Time required for part to reach minimum	Time required for part to regain volume	Time required for part to reach minimum and return to original volume	Reaction time	Time for recovery of the pulse wave	Number of reactions to stimulus	Mean reaction time
		<i>c. mm. per 5 cc. of part</i>	<i>c. mm. per 5 cc. of part</i>	<i>per cent</i>	<i>c. mm. per 5 cc. of part</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>		<i>seconds</i>
Light.....	24	7.7	5.4	30	31.5	10.6	28.2	38.1	3.26	34.0	7	3.42
Pin-prick...	31	8.5	5.2	39	35.1	8.7	27.2	35.8	2.95	25.3	19	3.18
Pistol shot...	28	8.3	4.8	42	30.7	10.2	24.5	36.8	3.07	34.9	15	3.76
Cold.....	33	7.6	5.1	33	29.8	9.2	18.0	26.1	3.07	22.8	10	3.56
Heat.....	24	9.2	5.6	39	35.1	10.2	18.4	28.0	3.04	28.2	5	2.86
Bell.....	29	9.0	6.2	31	29.8	11.1	22.7	27.5	3.33	24.0		
Shock.....	10	7.8	5.0	36	34.7	12.9	33.7	43.1	2.89	34.5	7	3.19
Mean.....		8.3	5.3	36	32.1	10.0	23.3	32.4	3.10	28.1		3.38

Statistical studies of the data:

Finger tips:

Reaction time: Mean = 3.12 ± 0.02 seconds

Standard deviation = 0.33 ± 0.01 second

Coefficient of variation = 10.6 ± 0.4 per cent

Toe tips:

Reaction time: Mean = 3.43 ± 0.04 seconds

Standard deviation = 0.49 ± 0.03 second

Coefficient of variation = 14.4 ± 0.87 per cent

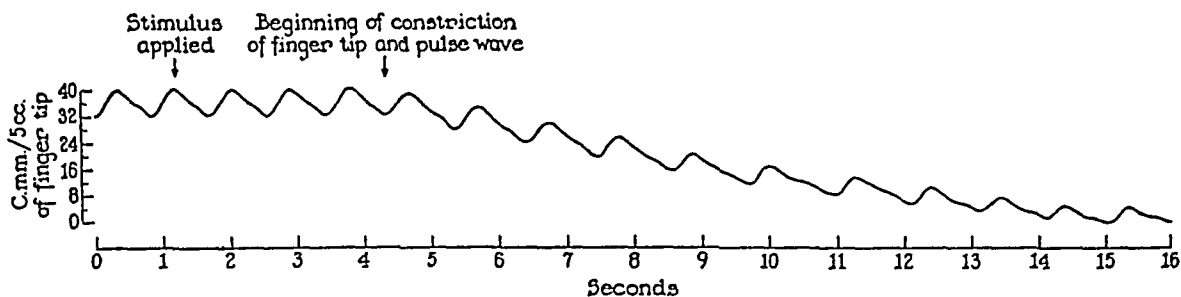


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covery was known to have taken place on return of the waves to their original volumes.

The reaction time to a stimulus was the time taken for a change in volume to begin (Figure 1). To study changes in volume, only the finger tips were measured. The measurements made were: (1) the initial volume of the pulse wave, (2) the volume of the pulse wave at its minimum, (3) the maximum change in "alpha" waves,³ (4) the time required for the "alpha" wave to reach a minimum, (5) the time required for the "alpha" wave to return to the original volume, and (6) the phase of the "alpha" wave when the stimulus was applied.

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RESULTS

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³ The reason for the use of quotation marks about the alpha will be explained later.

TABLE II

Mean values of various phases of the vascular response in the right index finger and right second toe tips of 12 resting patients with hypertension

Stimulus	Finger tips										Toe tips	
	Number of reactions to stimulus	Volume of pulse wave before stimulation	Volume of pulse wave when finger reached minimum volume	Decrease in volume of pulse wave	Maximum decrease in volume of part	Time required for part to reach minimum	Time required for part to regain volume	Time required for part to reach minimum and return to original volume	Reaction time	Time for recovery of the pulse wave	Number of reactions to stimulus	Mean reaction time
		<i>c. mm. per 5 cc. of part</i>	<i>c. mm. per 5 cc. of part</i>	<i>per cent</i>	<i>c. mm. per 5 cc. of part</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>		<i>seconds</i>
Light.....	17	8.6	5.2	40	28.3	8.9	14.9	24.0	3.39	28.1	6	3.53
Pin-prick...	26	6.4	3.5	45	23.0	8.6	16.5	26.0	2.81	22.8	6	2.77
Pistol shot..	17	8.4	4.9	42	26.7	8.4	22.2	30.6	3.00	21.1	6	3.07
Cold.....	34	6.4	3.9	39	24.7	9.5	18.2	27.4	2.89	26.0	12	3.26
Heat.....	34	7.6	4.6	39	29.7	9.1	19.7	28.9	2.91	29.1	15	3.33
Bell.....	31	8.1	4.7	42	26.7	8.3	17.5	25.4	2.78	23.2	11	3.33
Mean.....		7.4	4.4	41	26.4	8.85	18.2	27.2	2.92	25.6		3.25

Statistical study of reaction time

Finger tips: Total number of reaction times = 159

Mean = 2.94 ± 0.03 seconds

Standard deviation = 0.50 ± 0.02 second

Coefficient of variation = 17.0 ± 0.7 per cent

Toe tips: Total number of reaction times = 56

Mean = 3.24 ± 0.04 seconds

Standard deviation = 0.50 ± 0.03 second

Coefficient of variation = 15.4 ± 1.1 per cent

TABLE III

Mean values of various phases of the vascular response in the right index finger and right second toe tips of 11 resting senile subjects

Stimulus	Finger tips										Toe tips	
	Number of reactions to stimulus	Volume of pulse wave before stimulation	Volume of pulse wave when finger reached minimum volume	Decrease in volume of pulse wave	Maximum decrease in volume of part	Time required for part to reach minimum	Time required for part to regain volume	Time required for part to reach minimum and return to original volume	Reaction time	Time for recovery of the pulse wave	Number of reactions to stimulus	Mean reaction time
		<i>c. mm. per 5 cc. of part</i>	<i>c. mm. per 5 cc. of part</i>	<i>per cent</i>	<i>c. mm. per 5 cc. of part</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>		<i>seconds</i>
Light.....	3	11.4	9.8	14	29.4	11.2	23.1	34.4	4.14	34.4	2	4.67
Pin-prick...	8	9.4	6.8	28	12.6	9.1	15.0	24.5	3.92	31.8	1	4.46
Pistol shot..	6	9.7	7.4	24	25.0	11.8	25.0	36.3	4.06	42.7	4	4.09
Cold.....	28	9.3	6.7	28	22.2	13.0	20.1	32.2	3.72	29.0	10	3.85
Heat.....	17	8.1	6.0	26	19.9	11.4	19.2	33.8	4.16	30.5	5	4.59
Bell.....	18	10.5	7.2	31	30.0	12.5	33.6	46.2	3.58	37.8	6	4.50
Mean.....		9.4	6.9	27	22.8	11.9	22.1	34.7	3.84	33.4		4.23

Statistical study of the reaction time

Finger tips: Total number of reaction times = 80

Mean = 3.86 ± 0.04 seconds

Standard deviation = 0.58 ± 0.03 second

Coefficient of variation = 14.9 ± 0.8 per cent

Toe tips: Total number of reaction times = 28

Mean = 4.25 ± 0.07 seconds

Standard deviation = 0.54 ± 0.05 second

Coefficient of variation = 12.7 ± 1.2 per cent

tained from the senile and hypertensive patients was too small to allow for the compilation of a list showing significant differences in the times of reaction for the various stimuli. Such an attempt would be hazardous even in the normal group (178 responses), not only because of the relatively small number of responses to a single type of stimulus, but also because of individual variation in the sensitivity of subjects to stimuli applied directly to the skin.

Although there was much variation in time of reaction to successive stimuli, no correlation was established between reaction time and other factors, such as which limb of the spontaneous alpha wave was in process of formation at the time of the stimulus, frequency of application, and whether the subject was anticipating the application of a stimulus. If, as in the case of a few, subjects were asked to press a button to release a selected stimulus, their vessels became constricted when the button was pressed and no responses followed the sensory stimulus. Whenever the pulse wave was large, the response was constriction, involving, almost without exception, decrease in volume, both of the pulse wave and of the anatomical part ("alpha" wave). On the other hand, if a reaction could be detected, the initial size of the pulse wave did not seem to influence the reaction time. When the pulse was small, no clear reaction was evident, although occasionally it showed a slight decrease. In a small number of subjects, constriction of the anatomical part, as documented by a downward deflection in the "alpha" wave, was not accompanied by decrease in the size of the pulse waves. "Alpha" and pulse waves may accordingly respond independently to stimuli. Similar independence is observable in spontaneous variations (11).

In the finger tips, stimuli were effective in approximately 70 per cent of trials but in the toes, in 30 per cent only. The reason for this difference and that for the failure of so many stimuli is not known.

When reactions of the finger tip occurred at all, whether great or small, they did so after a reasonably uniform interval. But the time required to return to initial states varied widely (15 seconds to more than 3 minutes). This statement is applicable to all varieties of stimulation employed. For them all, the average recovery time in the

normal group was 32 seconds, the range being 26 seconds for cold and 43 seconds for shock. The average volume of the pulse wave immediately before the application of any one of the stimuli, that is to say the initial volume, was 8.3 c. mm.⁴ On stimulation, the average minimum reached was 5.3 c. mm. (a decrease of 36 per cent), the low point occurring after an average of 10 seconds. The average decrease in the volume of the part, as documented by the change in the "alpha" waves, was 32 c. mm. The average time for the recovery of the volume ("alpha" waves) was 23 seconds, whereas the pulse waves recovered in 28 seconds (Table I, Figure 1). This difference is further evidence that the two can vary independently, though usually they do not do so. If, instead of obtaining the average reaction time in the fingers of many persons, many measurements were made of a single subject, the results turn out to be identical. In one patient, for example, the mean reaction time was 3.08 ± 0.06 seconds in 47 responses. This figure is not significantly different from that of the entire group. The standard deviation was 0.58 ± 0.04 second and the coefficient of variation, 18.9 ± 1.4 per cent.

The respective mean values for the various phases of the vascular response of the finger tips other than reaction times in the hypertensive and senile subjects were: the volume of the pulse waves before application of each stimulus, 7.4 and 9.4 c. mm. per 5 cc. of part; the volume of the pulse waves when a finger reached its minimum volume, 4.4 and 6.9 c. mm.; the percentage of decrease in volume of the pulse wave, 44 and 27 per cent; the maximum decrease in volume of the part, 26.4 and 22.8 c. mm.; time required for the part to reach a minimum volume, 8.85 and 11.9 seconds; time required for the part to regain its volume, 18.2 and 22.1 seconds; and the time for the recovery of the volume of the pulse wave, 25.6 and 33.4 seconds.

The hypertensive subjects found it very difficult to relax. Changing from a room resembling a laboratory containing much apparatus and equipment to a bedroom of ordinary appearance (10) aided in bringing on relaxation. From this bedroom, all physiological apparatus was removed. The effect of change in the room was similar in

⁴ Changes in volume are given for 5 c. mm. of anatomical part.

senile patients, but practically no change was noticeable in the normal group which was recruited in great part from persons familiar with the procedures in laboratories.

Stimuli, such as bell and light, which could be used without the presence of an observer in the patient's room, were more frequently successful in eliciting responses than those which were applied by an observer in the room. With such a one present, the patients became tense and their vessels constricted at first, though relaxation took place after a short interval. Often it was not complete until the observer left the room. Senile subjects were less sensitive to this situation than hypertensive patients. When a stimulus was especially unpleasant to a hypertensive patient, he remained apprehensive and his blood vessels remained constricted for a long period of time, often for the duration of that particular observation. Such phenomena were less often encountered in senile subjects than in hypertensive patients. In fact, patients with hypertension were conspicuous in being unable to relax and in their tendency to be psychologically disturbed. The statements of patients on the tenseness of their sensations and their appearance of being ill at ease ran parallel with the occurrence of persistent vasoconstriction. If a reaction occurred, even if the vessels were constricted, the reaction time was essentially the same as when the vessels were dilated.

"Alpha" deflections documenting constriction of a part in response to stimuli differed from the alpha waves during spontaneous variations (3) in at least five ways: (1) After stimulation, vasoconstriction occurred more abruptly than during spontaneous variation. (2) After repeated stimulations at close intervals, the resulting downward deflections were more persistent than in the case of a series of spontaneous variations. (3) After stimulation, upward deflections did not occur. (4) In random samples of records of spontaneous variations taken for brief periods (3 seconds), downward deflections were rare. After stimulation, they occurred frequently in the fingers (65 to 70 per cent) and less frequently in the toes (30 per cent). (5) During periods of spontaneous variation, simultaneous, exactly synchronous downward deflections in both (right and left) fingers and toes were rare, though they were often

concordant. After stimulation, abrupt simultaneous downward deflections were the rule.

When there was reaction to stimulation, it resulted always in reduction in volume of the fingers and toes, never in dilatation. Nor was there ever increase in the size of the pulse waves. Reaction to heat did not differ from that of other stimuli. The reaction time was almost invariably the same in right and left index fingers and in right and left second toe tips, as has just been implied.

Two patients with hypertension were operated on.⁵ Three weeks after operation, the volumes of the pulse waves in the toes in both were larger and those in the fingers smaller than before operation. The vessels of the toes did not react to stimuli while those of the fingers reacted poorly.

DISCUSSION

In any study of the reactivity of the blood vessels, clearly an analysis of the pathway stimuli follow is important. In intact human beings, direct measurement of the time consumed in traversing each of the component parts of that pathway is impossible. There are involved, the time taken by the reception of the stimulus, the passage of the resulting impulse through the central nervous system, the effector pathway, and the local complex mechanisms which result in contraction of the blood vessels. The passage of the impulse from the point of reception and through the central nervous system occurs rapidly. The post-ganglionic fibers of the sympathetic nervous system, the ones most probably concerned in the transmission to the blood vessels, conduct impulses at varying rates in the order of $\frac{1}{2}$ to 1 meter per second. In order to attempt an explanation of the delay in reaction time in the case of the toes, different assumptions may be entertained. If it can be assumed that the local mechanisms involved in the contraction of the blood vessels in the fingers and toes are alike, the added distance impulses must travel to the toes accounts for the delay of the order of 0.30 second. It is possible on the other hand that the major part of the delay is not consumed in the transmission of impulses over paths of different lengths but is due to differences in local mechanisms in

⁵ The operation consisted of section of the sympathetic nerves from the 9th dorsal to the 1st lumbar, together with excision of the celiac and aortic-renal ganglia on both sides.

fingers and toes. It seems likely now that the difference in length of pathways is sufficient to account for the delay to the toes. The notion is not yet to be excluded however that local differences, as for example a mere matter of the number of units of nerves or receptors, may be concerned in the result. There is a third possibility; delay may result from the pathway within the spinal cord over which an impulse must pass when received from the upper part of the body, before it reaches that level of the cord from which it emerges to proceed to the lower extremity. There is a final consideration. There is often detected a difference in reaction time, in multiples as great as 2. These differences are now not understood. They do not depend on any factors which it has been possible to identify. That variations from subject to subject or from time to time in the same subject are dependent on variations in the conduction rates of the impulses through nerves is unlikely; it is more likely due to variations in the blood vessels. But on such problems, no definite data have been established.

Since the method now employed measures the constriction of the entire finger or toe tip, which of the many types of blood vessels reacted first or accounted for most of the constriction cannot be known. The fact that in some subjects the reaction resulted in a reduction in volume without concomitant reduction in the size of the pulse wave tends to indicate that vessels other than arteries and arterioles also react to the stimuli. The reaction times found in these studies were

never as great as those reported by Fano (7) who found values as high as 7 seconds.

The range of variation in the time occupied in reacting to a stimulus was moderately wide, but not nearly as wide as that required to bring about maximum constriction of a part. Many factors, most of them unknown and beyond control, play a part in influencing both the time and the degree of the total response. The importance of the immediate environment has already been emphasized. And it has already been remarked that subjects vary from moment to moment. When all the causes of variation are taken into account, the fact remains that there is great regularity in the physiological control of blood vessels.

The question has been weighed whether impulses which traverse the brain have reaction times different from those which may pass only through the spinal cord. The mean reaction times in the fingers of normal subjects for light, bell, and pistol shot were 3.26, 3.33, and 3.07 seconds. Those for shock, pin-prick, cold, and heat were 2.89, 2.95, 3.07, and 3.04 seconds (Table I). At first glance, it appears that a reflex passing through the brain experiences a delay. The fact that both pistol shot and cold consume the same time is not easily reconciled with this view.

The vascular responses to the stimuli were widely distributed, having occurred almost simultaneously in fingers and toes on both sides. There was, in addition, definite slowing of the heart rate (Figure 2).

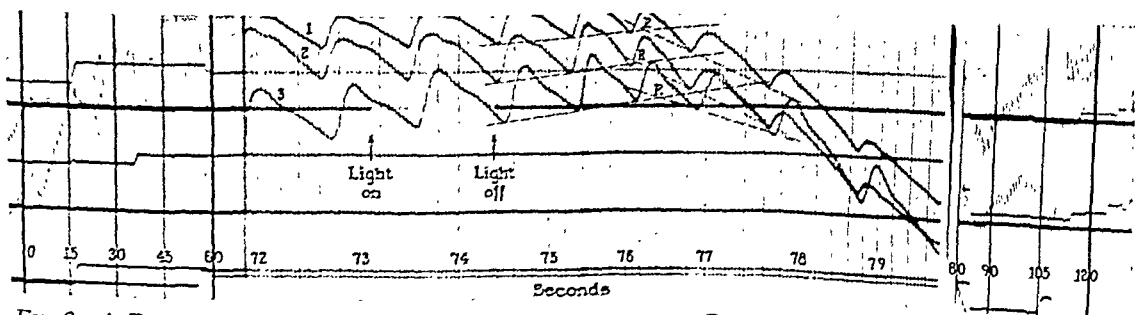


FIG. 2. A REPRESENTATIVE ORIGINAL RECORD OF THE TIPS OF THE RIGHT, 1, AND LEFT, 2, INDEX FINGER AND THE RIGHT, 3, SECOND TOE

These are simultaneous records. From about the 72nd to 80th seconds, the camera speed was approximately 6 cm. per second while otherwise the speed was about 8 cm. per minute. *R* indicates the point at which vasoconstriction occurred. This point was found at the intersection of a line which joined the foot points of the last 4 pulse waves just before the volume of the part changed and another one through the first few pulse waves just after vasoconstriction began. The time of reaction was measured from the point of the application of the stimulus to the point *R*. Bradycardia is evident during the period of vasoconstriction.

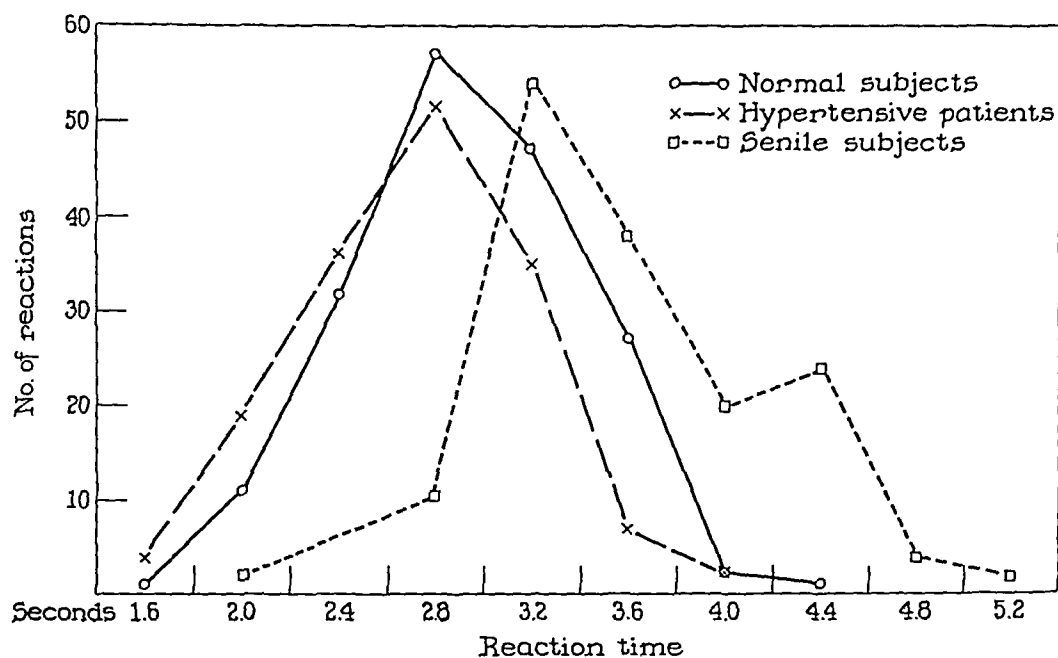


FIG. 3. DISTRIBUTION CURVES GIVING THE REACTION TIMES OF NORMAL, HYPERTENSIVE, AND SENILE INDIVIDUALS

These curves overlap. The curve of the hypertensive patients is farther to the left than that of the normal persons; that of the senile subjects, relatively farther to the right. This curve (senile) has a wider distribution than the other two.

The degree of vasoconstriction, suddenness of constriction, duration of constriction, time required for the constriction to reach its limit, and the time required for recovery were not materially different in the case of any of the stimuli used (Table I). There was no method available according to which normal subjects could be grouped on the basis of any aspect of their responses.

It was noticed that some individuals were made much more apprehensive by certain stimuli than by others. In some subjects, the electric shock, the pistol shot, or even heat or cold were so distressing as to prevent them from relaxing normally. The electric shock produced apprehension most often. After the first observation, those stimuli which produced psychic discomfort were recognized. On the next observation, the subject was informed at the beginning of the study that the offending stimulus would not be employed. Invariably it was found that relaxation then occurred more quickly and completely. During some observations, a subject would relax and would respond to stimuli which were not disagreeable but would, once one was found to be so from a psychological point of view, react so that his blood vessels contracted but would not recover within a reasonable period of time. The vessels

remained tightly constricted, the patient became afraid, and no further reactions of the blood vessels were detected, regardless of the stimulus applied. It was found that the stimuli, the room (10), the entrance of a strange observer, and many other factors which are usually regarded as innocuous would often disturb the patient sufficiently to prevent satisfactory relaxation. It is impossible in such a study, or in all probability in any other type of physiological study on the vascular system in conscious human beings, to over-emphasize the importance of psychological factors. Emotional or ideational stimuli, such as result from conversations involving sex, business, or matters of personal concern, elicited vascular reactions similar to those described in the case of mechanical stimuli.

The measurements demonstrate that the times of reaction in the fingers of senile subjects and of hypertensive patients are reasonably close to those of normal persons (3.12), but that those of the senile ones are longer (3.86), and those of the hypertensive ones, shorter (2.94) (Figure 3). The difference, 0.18 second, between normal persons and patients with hypertension is statistically significant. It should not be over-emphasized, nevertheless, because these measurements are not

accurate beyond ± 0.05 second. Aside from the differences in reaction time, other aspects of the nature of the vascular response are distinctive. The slope of the curve is more precipitous, the state of maximum constriction is attained more quickly, the reaction takes place to a greater degree, and is over more quickly, than in the normal people. If, instead of a stimulus which is innocuous, psychologically, and which has no psychological persistence, stimuli occurred or were applied which were more painful or more far-reaching in influence, the continuance of constriction would exhibit far greater duration. This observation suggests that, in hypertensive patients, the impact of usual occurrences, not ordinarily regarded as harmful, in daily life result in effects upon their vascular systems of a degree and a duration beyond that experienced by normal persons. Unfortunately, it has been impossible to measure changes in the blood pressure. No satisfactory apparatus free from the danger of occasioning psychological stress that would serve the current purpose is now available.

In the senile subjects, clearly the reaction times of the fingers and toes were much longer than in normal or hypertensive persons. The reaction takes place, furthermore, not only more slowly but the entire response is slow to reach its maximum, slow to recover, the degree of the response is less, and the entire response has the appearance of less vigor. The distribution curves of the times of reaction of the finger tips of the three groups of subjects illustrate these differences. The means in these three groups are distinct. There is a considerable amount, nevertheless, of overlapping of these values. The shapes of the distribution curves are such that the hypertensive curve which is to the left exhibits a fair sized area of overlapping while that of the senile group to the right, is much wider, and so comes to coincide less with the normal curve. As quantities, though significant, these differences are small; as qualities to be perceived clinically, they are very distinct.

The reactions in the toes of the hypertensive patients and senile subjects took place 0.30 and 0.39 second later, respectively, than in the fingers. These differences indicate no difference statistically among the three groups when compared with the value of 0.31 in normal individuals.

By good fortune, it was possible to learn more intimately the path which these impulses traversed. The observation was made possible by the 2 patients with hypertension who were operated upon. Their sympathetic nerves, after they were cut, naturally failed to convey impulses and so could not serve as the efferent limits of reflex arcs set in motion by sensory stimuli. Expected responses, therefore, did not occur.

SUMMARY

The mean reaction times in the tips of the fingers in normal (3.12) and in senile persons (3.86) differ from those in hypertensive patients (2.94), being most rapid in the hypertensive and slowest in the senile persons. In the tips of the toes, the general arrangement is the same, being fastest in hypertensive subjects (3.24) and slowest in the senile (4.25). In the toes, the delay (beyond the fingers) is of the same order of magnitude in each of the three groups. This can be accounted for on the basis of the time required for the efferent impulses to traverse the additional length of post-ganglionic sympathetic fibers in order to reach the toes. The stimuli used were diffuse light, heat, cold, pin-prick, sudden loud noise (pistol-shot), and electric shock. There was no significant difference in the normal group among the stimuli used in the reaction time or in any part of the total vascular response, such as time for the vasoconstriction to reach a maximum, degree of change in the volume of the pulse wave, time for recovery, and suddenness of response. It was not possible to group persons on the basis of their reactions to the stimuli.

The stimuli, light and bell, which were applied while subjects were alone were more satisfactory than those which, when applied, necessitated the presence of an observer. Psychological factors, often apparently very mild, influenced the responses significantly, which indicated the extreme importance of recognizing them during peripheral vascular studies on conscious human beings.

No correlation was found, provided a reaction to the stimulus occurred, between the reaction time and the state of the vascular bed of the part. The reaction time was essentially not affected by the fact that the vascular bed was already in a contracted or dilated state or was constricting or

dilating when the stimulus was applied. This was not the case concerning the degree of change in volume of the vascular bed during the response. The more constricted the vascular bed at the time of stimulation, the less change in volume.

In general, the data strongly suggest that reaction time was more rapid, the vascular response occurred more suddenly and to a greater degree and was over more rapidly, in hypertensive than in normal persons.

In the senile subjects, the reaction time was less rapid than normal and the vascular response occurred more slowly, to a less degree, and the recovery was much slower.

The reason for these differences is unknown. These differences can be owing to changes in the vessels themselves or in factors outside the vessels such as the nervous system or in chemical states which influence the vessels.

BIBLIOGRAPHY

1. Burton, A. C., The range and variability of the blood flow in the human fingers and the vasomotor regulation of body temperature. *Am. J. Physiol.*, 1939, 127, 437.
2. Hertzman, A. B., and Dillon, J. B., Selective vascular reaction patterns in the nasal septum and skin of the extremities and head. *Am. J. Physiol.*, 1939, 127, 671.
3. Burch, G. E., Cohn, A. E., and Neumann, C., A study by quantitative methods of the spontaneous variations in volume of the finger tip, toe tip, and postero-superior portion of the pinna of resting normal white adults. *Am. J. Physiol.*, 1942, 136, 433.
4. Evans, C. L., The physiology of plain muscle. *Physiol. Rev.*, 1926, 6, 358.
5. Lapicque, L., and Lapicque, M., Chronaxie des vaisseaux et des cellules pigmentaires chez la grenouille. *Soc. Biol., Paris*, 1924, 91, 267.
6. Fredericq, H., Chronaxie. *Physiol. Rev.*, 1928, 8, 501.
7. Luciani, L., *Physiologie des Menschen*. Fischer, Jena, 1905, vol. 1, pp. 290-291.
8. Hines, E. A., and Brown, G. E., A standard test for measuring the variability of blood pressure: Its significance as an index of the prehypertensive state. *Ann. Int. Med.*, 1933, 7, 209.
9. Turner, R. H., Studies in the physiology of blood vessels in man. Apparatus and Methods. I. A sensitive plethysmograph for a portion of the finger. *J. Clin. Invest.*, 1937, 16, 777.
10. Neumann, C., Cohn, A. E., and Burch, G. E., A study of the influence of the character of an examining room on the peripheral blood vessels of normal, hypertensive, and senile subjects. *J. Clin. Invest.*, 1942, 21, 651.
11. Neumann, C., Cohn, A. E., and Burch, G. E., A study of the relationship between pulse and alpha waves of the tips of the fingers and toes of five adults. *Am. J. Physiol.*, 1942, 136, 448.

BLOOD FLOW IN THE HAND AND FOREARM AFTER PARAVERTEBRAL BLOCK OF THE SYMPATHETIC GANGLIA. EVIDENCE AGAINST SYMPATHETIC VASODILATOR NERVES IN THE EXTREMITIES OF MAN

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Many of the blood vessels of the extremities are under reflex control. Numerous stimuli, such as cooling the body, pain, loud noise, or deep inspiration, produce reflex vasoconstriction. Heating the body causes reflex vasodilatation. The fact that either pre- or post-ganglionic sympathectomy eliminates these vasoconstrictor and vasodilator responses demonstrates that the sympathetic nerves contain the efferent limb of the reflex arc for both types of response (1, 2). These vasomotor reflexes which are mediated through the sympathetic nerves could be explained equally well by either of the following hypotheses: (a) that vasoconstriction is an active process resulting from reflex sympathetic stimulation, and vasodilatation is a passive phenomenon resulting from inhibition or lack of sympathetic activity; (b) that both vasoconstriction and vasodilatation are active processes resulting from reflex activity. The latter hypothesis assumes that part of the reflex vasodilatation results from inhibition of active vasoconstriction, but that vasodilatation beyond that point is an active process resulting from reflex stimulation of the vasodilator nerves. This hypothesis gained support from the demonstration that the sympathetic nerves to the extremities contain both adrenergic and cholinergic fibers. The nerves supplying the sweat fibers are cholinergic, but anatomically they form a part of the sympathetic system. Might it not be possible that vasodilator, cholinergic fibers also coursed through the sympathetic ganglia and nerves?

Lewis and Pickering observed that heating the body was the most satisfactory means of causing neurogenic vasodilatation in the extremities of both normal subjects and patients with Raynaud's disease, and that this response to body heating occurred only in the presence of intact sympathetic innervation (1). They noted that in normal subjects whose skin temperature had previously been

lowered by exposure in a cold room, blocking the ulnar nerve caused vasodilatation and warming of the anesthetized fingers. In two of their patients with Raynaud's disease, block of the ulnar nerve did not cause the fourth and fifth fingers to warm. Heating the body produced normal vasodilatation in these fingers. They concluded: "To sum up, it is found in cases of Raynaud's disease exposed to suitable room temperature that ulnar anesthesia, with its inhibition of vasoconstrictor tone, fails to release the vascular spasm, but that under the same conditions, warming the body relaxes the vessels. The last effect occurs through sympathetic paths, failing to occur after sympathectomy, but as it is not due to simple inhibition of vasoconstrictor tone, being, in fact, prevented by anesthetization of the mixed nerve, it must be attributed to active vasodilatation." These experiments are not conclusive because no evidence was obtained to show that the block of the ulnar nerve had actually inhibited the vasoconstrictor impulses to the vessels supplying the fourth and fifth fingers. It has been found that it is not always safe to rely on anesthesia alone as a sign that all sympathetic fibers to the area are blocked (3). Lewis and Pickering (1), estimating changes in blood flow from changes in skin temperature, were unable to obtain evidence of vasodilator nerves in normal subjects. They believed that these negative experiments were not conclusive because changes in blood flow are not always reflected by changes in skin temperature. They pointed out that the loss of vasoconstrictor tone raised the temperature of the part to such a degree that further active vasodilatation had little chance of causing a further rise in skin temperature.

Grant and Holling (3) studied the blood flow in the forearm during intensive body heating. They found that the blood flow increased, and that this increase was dependent on the integrity

of the sympathetic nerves. Local nerve block, however, caused the anesthetic skin to become pale and cool. "Moreover, if the skin is first flushed and warmed, and kept so by heating the body, and a cutaneous nerve is then blocked (the circulation to the hand being arrested), the area then becomes anesthetic and ceases to sweat, pales and cools." These authors concluded that there are two means of defense against a rise of body temperature. "The first is brought into action by relatively gentle heating and consists chiefly of a dilatation of the arteriovenous anastomoses in the extremities, caused by inhibition of vasoconstrictor tone. The second is added when the heating is more intense and consists mainly of a general dilatation of the cutaneous vessels associated with sweating, both of which are caused by stimulation of the sympathetic nerves." These experiments, too, suggest that the sympathetic nerves carry vasodilator fibers, but they are not conclusive. No data are given to show that novocainization of the peripheral nerves had produced complete sympathetic paralysis. Lack of sweating of the anesthetized skin was noted, but recent investigations have shown that sweating may be markedly decreased without interfering with other functions of the sympathetic nerves (4). The authors point out that the circulation in the forearm is considerably increased immediately after sympathetic ganglionectomy, and they are unable to account for the difference between the effect of blocking a mixed nerve to a part of the limb and the effect when the sympathetic nerves supplying almost the whole limb are blocked.

The fact that heating the body produces a greater blood flow in the normal extremity than in the extremity which has been sympathectomized for some time cannot be used as evidence for the existence of vasodilator nerves (1). It has been repeatedly shown that there is an increase in tone in vessels deprived of their sympathetic nerve supply. If the comparisons between the blood flow in the normal and in the sympathectomized extremities are made after several days, the compensatory changes in the sympathectomized part make interpretation of the results difficult. The point at issue is whether, immediately after sympathetic section, the blood flow in the normal extremity can be increased by reflex stimulation to

a level above that in the sympathectomized extremity.

It is possible to study the problem of whether vasodilator fibers are present in the sympathetic nerves supplying the extremities by determining the blood flow in the upper extremity before and after novocainization of the sympathetic ganglia supplying the part. The novocain blocks both vasoconstrictor and vasodilator stimuli. If vasodilatation is a passive process resulting from inhibition of reflex vasoconstriction, then blocking all vasoconstrictor impulses will cause as great a degree of vasodilatation as can be produced through reflex response to heating the body. On the other hand, if the sympathetic nerves carry both vasoconstrictor and vasodilator fibers, paralysis of both of these will produce some increase in blood flow because of removal of the vasoconstrictor impulses, but it will not produce as great an increase in blood flow as will reflex vasodilatation from heating the body, because the vasodilator impulses will also be blocked.

METHOD

These experiments were carried out on a normal, paid volunteer. The blood flow in the hand and forearm was measured by plethysmographic methods (5, 6). When the blood flow in the forearm was determined, the circulation to the hand was occluded by a tourniquet, distal to the forearm plethysmograph (7, 8).

The subject sat upright in a chair with the arms resting on a table in front of him. The parts to be studied were placed in the plethysmograph and surrounded by water of the desired temperature for at least 30 minutes before the experiment was started. Control flows were taken.

The sympathetic ganglia supplying the right upper extremity were then paralyzed by the paravertebral injection of novocain. The spinous processes of the 7th cervical and upper 5 dorsal vertebrae were marked with a skin pencil and scratches were made 4 cm. lateral to each spinous process. The skin over the region was then disinfected, and wheals were raised by injecting 1 per cent procain hydrochloride into the skin surrounding each scratch. A 3 inch, No. 22 gauge needle was inserted into the wheal opposite the 3rd spinous process, and advanced perpendicular to the skin, toward the lower border of the rib, until it impinged in the general region of the tubercle. After contacting the rib, the needle was withdrawn slightly, a 2 cc. syringe full of 1 per cent procain was attached, and the needle was advanced slowly in a transverse plane at an angle of about 30 degrees to the sagittal plane of the body. Procain was infiltrated along the inferior margin of the costovertebral joint, the neck, and the head of the rib, so that there was no lasting

TABLE I
Summary of the experimental data on the hand

	Temperature of water in plethysmograph		Blood flow in cc. per minute per 100 cc. of tissue		Remarks
	Right	Left	Right	Left	
Resting	30° C.	30° C.	1	1	Room temperature: 21° C. Body exposed.
Reflex vasodilatation induced by immersing legs in hot water at 43° C.	30° C.	30° C.	8	11	The subject is warm all over and has begun to sweat. Vasodilatation is not complete.
After right paravertebral block. Heating of legs continued	31° C.	31° C.	32	0-?	On the left, the vasoconstrictor effect of pain has overcome the vasodilator effect of heating the body. There is no vasoconstrictor effect on the right because of the paravertebral block.
After recovery from paravertebral block. Heating of legs continued ..	31° C.	31° C.	13	13	Blood flow in both hands gradually rising as heating of the body continues.
Temperature of water surrounding right hand raised to 43° C. Heating of legs continued	43° C.	31° C.	34	19	Full vasodilatation in the right hand from a combination of local heat and heating the body did not produce a significantly greater blood flow than was produced by block of the sympathetic ganglia.

discomfort. The needle was inserted until it struck the body of the vertebra. In each instance, the patient described paresthesias along the intercostal nerve as the needle was advanced the last 1 or 2 cm.

The 4th and 5th intercostal spaces were needled in succession, and then the 2nd and the 1st. Finally, a needle was inserted opposite the 7th cervical spine in a direction parallel to the needle in the first space. Because of the position of the patient, the upper 3 needles had to be angulated downward slightly as they skirted the neck of the ribs. Ten cc. of alkaline procain (9) were injected slowly in each interspace. After sympathetic paralysis developed, as evidenced by vasodilatation and the development of Horner's syndrome on the injected side, the needles were removed.

Reflex vasodilatation was produced by immersing the legs in hot water (43° C.). Heating the body does not produce maximal vasodilatation in the hand when the water in the plethysmograph is maintained at 30° C. (8). Therefore, when the full degree of vasodilatation which could be induced by heat was desired, the part under observation was surrounded by hot water. When the water was in contact with the skin, as in the hand plethysmograph, the temperature was kept at 43° C. When the water was separated from the skin by a thin rubber membrane, as in the forearm plethysmograph, the temperature was maintained at 46° C.

RESULTS

Blood flow in the hands. The data from this experiment are summarized in Table I. In this paper, the blood flow is recorded as cc. of blood flow per minute per 100 cc. of tissue enclosed within the plethysmograph. With the temperature of the water in the plethysmographs at 30° and the arms, chest, and lower extremities exposed, the blood flow in each hand was 1 cc. per minute per 100 cc. of tissue (Figure 1-A). Room temperature was 21° C. The lower extremities below the knees were then immersed in water at 43° C. Body heating by this means was continued throughout the entire experiment. Partial reflex vasodilatation occurred and the blood flow in the hands increased, rising to a level of 8 cc. in the right, and 11 cc. in the left (Figure 1-B). At this time, normal vasomotor activity, which is dependent on the integrity of the sympathetic nerves, was demonstrable in both hands (Figure 2-A). The sympathetic ganglia supplying the right upper extremity were then paralyzed by right paraverte-

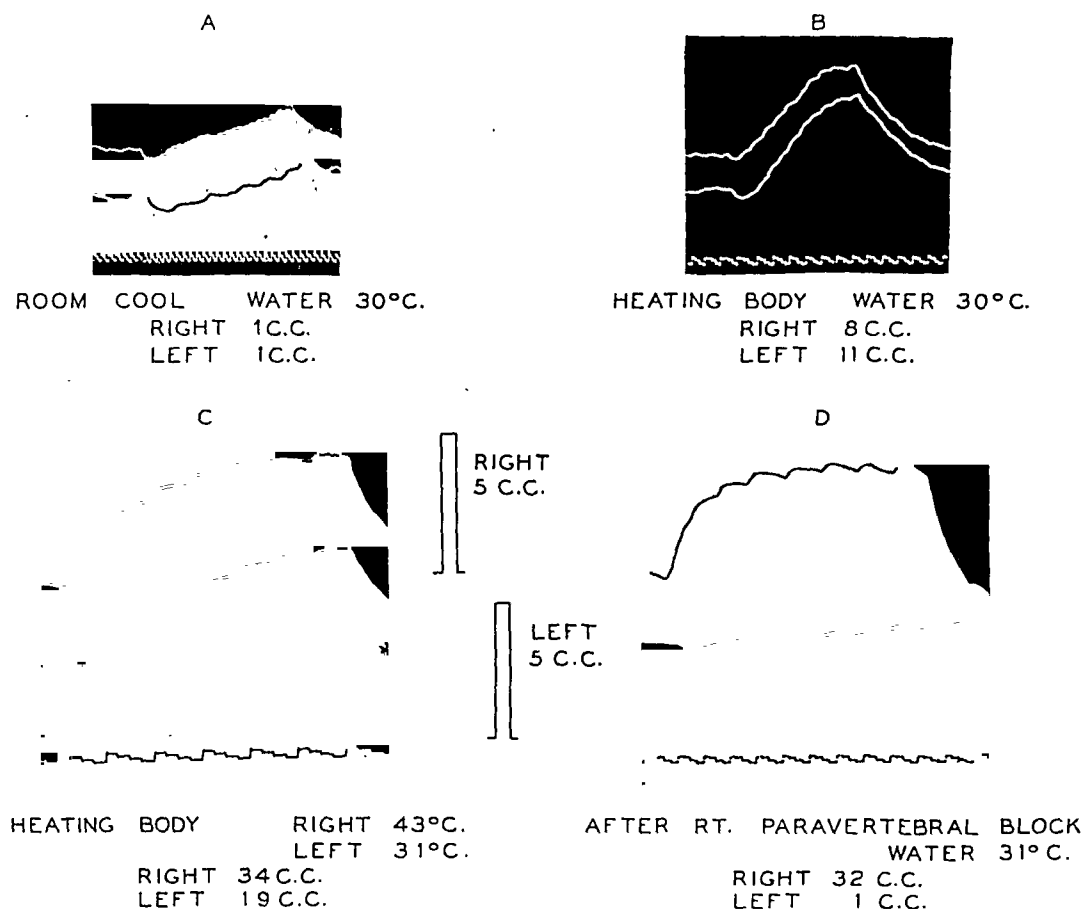


FIG. 1. BLOOD FLOW IN THE RIGHT (UPPER) AND LEFT (LOWER) HANDS

Recorded in cc. of blood per minute per 100 cc. of hand. A. Control. Body exposed, room temperature 21° C., water in plethysmograph at temperature of 30° C. B. After immersing lower legs in water at a temperature of 43° C. The blood flow has increased in both hands. C. Legs still in water at 43° C. Water about right hand at temperature of 43° C. Blood flow increased in both hands but greater in the one surrounded by the hot water. D. After right paravertebral block. Water about both hands at temperature of 31° C. Note that blocking the sympathetic nerves produces as great an increase in blood flow as the combination of local heat and heating the body (Figure 1-C). The pain produced by inserting the needles for the paravertebral block has caused marked vasoconstriction in the left hand.

bral injection of novocain. The pulse waves in the right hand showed a marked increase in size, and the vasomotor activity which was present before the block disappeared completely (Figure 2-B). A deep breath, or pinching the skin, no longer produced vasoconstriction in the right hand. There was a marked Horner's syndrome on the right. The skin of the right face and arm was warm and flushed, and the veins, full. There was no sweating. Note that the left hand now showed no spontaneous variations in volume, but for a different reason. The pain caused by putting the needles in place for the paravertebral block had produced generalized vasoconstriction in all parts

of the skin except where the sympathetic nerves had been paralyzed. The skin of the left arm was cold and clammy. The vessels of the left hand were fully constricted, and the blood flow, very slow. The vasoconstrictor effect of pain had completely overcome the vasodilator effect of heating the body. The blood flow in the right hand was now 32 cc. (Figure 1-D); that in the left hand was too low to be measured for some minutes, but it gradually increased as the effects of the pain decreased. As the effect of the novocain wore off, the blood flow in the right hand gradually decreased. Because of the continued body heating, the blood flow in both hands remained somewhat

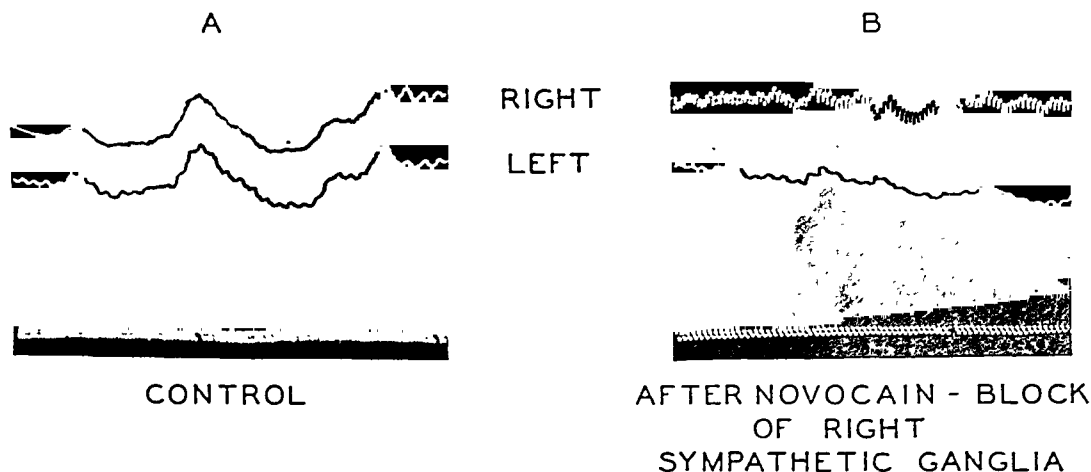


FIG. 2. VASOMOTOR RESPONSES IN THE RIGHT (UPPER) AND LEFT (LOWER) HANDS

A. Control. Normal vasomotor activity in both hands. B. After right paravertebral block. The arterial pulsations are very marked in the right hand, and there is no evidence of vasomotor activity. There is marked vasoconstriction in the left hand because of the pain caused by inserting the needles for the block.

above the level present before the block. Spontaneous vasomotor activity was now present in both hands.

This portion of the experiment demonstrated that the blood flow in the right hand, already increased by reflex vasodilatation from heating the body, was further increased by blocking all the sympathetic nerves to the part. It was obvious, however, that complete vasodilatation was not present in the hand before the paravertebral block. It has been demonstrated that, in the hand, complete vasodilatation is not produced by heating the body if the temperature of the water surrounding the hand is maintained at 30° C. (8). The maximal vasodilatation that can be induced by heat will occur if the water is maintained at a

temperature of 43° C. Therefore, in order to compare the effect of paravertebral block with the full vasodilator effect of heat, it was necessary to raise the temperature of the water surrounding the right hand.

The water about the right hand was heated to 43° C., that about the left hand remained at 31° C. Twenty minutes later blood flows were recorded in both hands; that in the right was 34 cc.; that in the left, 19 cc. The blood flow in the left hand had risen because of the continued body heating produced by having 3 extremities immersed in water at 43° C. The flow in the right hand had increased above that in the left hand because of the combined effect of local heat and body heating. It is significant that this combination

TABLE II

Summary of experimental observations on right forearm

	Temperature of water in plethysmograph	Blood flow in cc. per minute per 100 cc. of tissue	Remarks
Resting	31° C.	2	Room temperature: 22° C. Block satisfactory, as shown by hot, dry right hand with rapid blood flow. The left hand was moist and cold.
After right paravertebral block.....	31° C.	12	
Recovery stage	31° C.	2	The temperature was raised to 46° C. rather than 43° C. because in the forearm plethysmograph the water is separated from the skin by a thin rubber membrane.
Temperature of water surrounding right forearm raised to 46° C.....	46° C.	12	

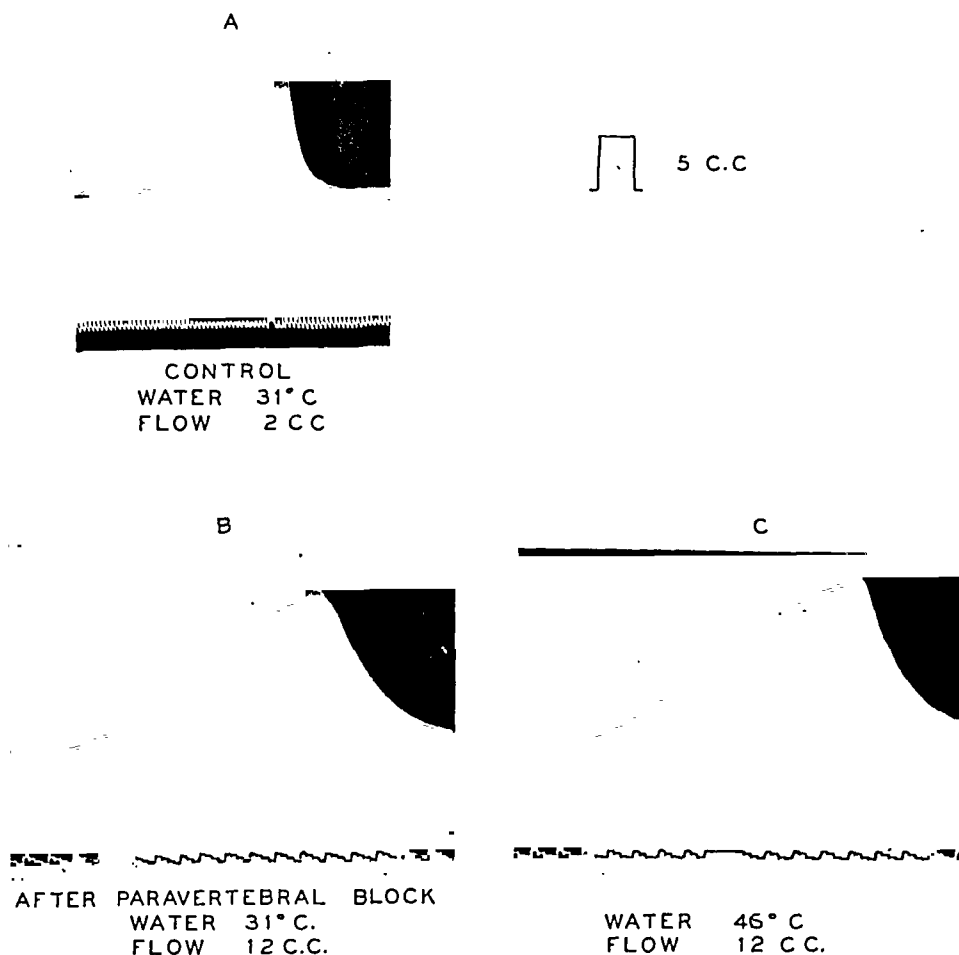


FIG. 3. BLOOD FLOW IN THE RIGHT FOREARM

Recorded as cc. per minute per 100 cc. of forearm. Circulation to the hand occluded. A. Control. Water in plethysmograph at 31° C. B. After paravertebral block. Water in plethysmograph at 31° C. Note marked increase in blood flow. C. After water in the plethysmograph had been at temperature of 46° C. for forty minutes.

of local and body heating caused no greater vasodilatation in the right hand than did block of the sympathetic nerve supply. It was, therefore, concluded that there were no vasodilator fibers in the sympathetic nerves supplying the hand, because removal of all sympathetic impulses produced complete vasodilatation.

Blood flow in the forearm. Because the vascular reactions of the forearm differ somewhat from those of the hand (7, 8, 10), it was still possible that the sympathetic fibers supplying the forearm might contain vasodilator fibers. Therefore, at another time, the response of the vessels of the forearm after blocking of the sympathetic nerve supply was studied (Table II).

The subject sat upright in a chair, with the right forearm in a plethysmograph. The water in the plethysmograph was maintained at a temperature of 31° C. The resting blood flow was 2 cc. per

minute per 100 cc. of forearm. The sympathetic nerves to the right upper extremity were then blocked by the paravertebral injection of novocain. This produced a marked Horner's syndrome on the right. The skin of the right face and right arm became warm, flushed, and dry. The veins were full. The skin of the remainder of the body was pale, cold, and covered with perspiration from the pain which was produced by putting the needles in place for the injection. The blood flow in the right forearm was 12 cc. after the block. As the effect of the novocain wore off the blood flow returned to the control level. The temperature of the water in the plethysmograph was then raised to 46° C. Forty minutes later the blood flow in the forearm was 12 cc. As in the hand, paralysis of the sympathetic nerves had produced as great vasodilatation as did the application of local heat. It was not considered necessary to

determine the blood flow after body heating because it has been shown that the application of local heat to the forearm produces more complete vasodilatation than is obtained from heating the body (8).

COMMENT

This investigation was carried out on only one subject. In each set of experiments, data were collected before, during, and after the paravertebral block. Because of the nature of the investigation, it was possible to make repeated observations during each phase of the experiment. It is felt that this type of carefully planned experiment, in which the subject serves as his own control, is more valuable than less complete observations on several subjects.

Other investigators have not produced as great a degree of vasodilatation from paravertebral block as they have from heating the body (6, 11). They made no attempt, however, to determine whether the injection of novocain had completely destroyed all evidences of sympathetic activity. Their results are comparable to those obtained by us while the effect of the novocain was wearing off. Partial block produces some increase in blood flow, but not as much as is caused by body heating. It has been previously shown that the majority of the vessels in the hand are fully dilated by local heat (8). The fact that blocking sympathetic impulses to the hand produces as complete vasodilatation as does heating the part indicates that most of the vessels in the hand are under the influence of the sympathetic nervous system. As will be pointed out below, the forearm differs from the hand in this respect.

In the forearm, blocking of the sympathetic ganglia caused an increase in blood flow to the same level as that produced by local heat. It is known that in the forearm, local heat produces as great, or greater, vasodilatation as that produced by heating the body (8). Neither local heat, nor a combination of local heat and body heating, produces maximal vasodilatation in the forearm (8). Exercise causes much greater vasodilatation than occurs as a reflex response to heating the body, or than was produced in this subject by block of the sympathetic nerves. This indicates either that, in contrast to the hand, many of the vessels of the forearm are not under the control of the sympa-

thetic nervous system, or that heat is not an adequate stimulus for producing reflex vasodilatation in the forearm. Experimental observations have shown that heating the body is the strongest known stimulus for reflex vasodilatation of the vessels of the forearm. The vasodilatation induced by arterial occlusion, exercise, and epinephrine is not reflex in origin. As the vessels of the skin of the hand have been shown to be under sympathetic control, it is logical to assume that the vessels of the skin of the forearm are also under sympathetic control, and that the difference in the reaction of the vessels of the hand and of the forearm to heat and sympathetic block is explained by the greater mass of muscle in the forearm. These observations suggest that the sympathetic nervous system plays little part in regulating the blood supply to the muscles.

Grant (12) has demonstrated that the local vascular effects of exercise are independent of the sympathetic nerves. Kunkel, Stead, and Weiss (8) concluded that local heat to the forearm caused much greater dilatation in the vessels of the skin than in the vessels of the muscles. They noted that though the resting blood flow could be greatly varied by raising or lowering the temperature of the water surrounding the forearm, vigorous exercise of the forearm produced approximately the same increase in blood flow above the resting level. It has also been shown that arterial occlusion produces a much greater blood flow in the forearm than occurs after reflex dilatation from heating the body, or from a combination of body heating and local heat to the forearm (8, 10). This again is in sharp contrast to the findings in the hand and foot, where local heat produces more nearly maximal vasodilatation. The reactive hyperemia in the forearm produced by the release of arterial occlusion is not altered by sympathectomy (10).

Patients with destruction of a lateral portion of the medulla oblongata, from thrombosis of the posterior inferior cerebellar artery, frequently show partial sympathetic paralysis. In certain of these patients, the vessels of the extremities on the side of the lesion are not able to constrict fully when the body is cooled, but they dilate normally when the body is heated (4). They resemble cases of unilateral peripheral sympathectomy in

that the involved hand is the warmer one when the body is exposed in a cool room, but they differ from the unilateral sympathectomy in that warming the body produces as great an increase in blood flow in the involved hand as it does in the normal hand. The induction of this type of paralysis of vasoconstriction without disturbance of vasodilatation would be ideal in the treatment of Raynaud's disease. Heretofore, there have seemed to be two equally plausible explanations for this dissociation between vasoconstriction and vasodilatation: (a) that the sympathetic nerves to the extremities contain both vasoconstrictor and vasodilator fibers and that the medullary lesion destroyed the central connections of the vasoconstrictor fibers without injuring the tracts concerned with vasodilatation; (b) that the sympathetic nerves contain only vasoconstrictor fibers, vasodilatation being simply inhibition of vasoconstriction, so that partial destruction of the central connections of the vasoconstrictor fibers interfered with the active process of vasoconstriction without disturbing the passive vasodilatation resulting from inhibition of vasoconstriction. The experimental data reported here indicate that the latter is the correct explanation. It is of interest that with the medullary lesion the remaining connections of the vasoconstrictor nerves are sufficient to prevent the vessels of the extremities from exhibiting the increase in tone which follows either pre- or post-ganglionic sympathectomy, though they are not capable of producing normal vasoconstriction on cooling the body. Therefore, inhibition of vasoconstriction still allows full normal vasodilatation to occur.

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(6) The fact that neither heating the forearm nor injection of the sympathetic ganglia with novocain produces maximal dilatation in the forearm indicates that many of the vessels of the forearm are not under control of the sympathetic nervous system. It is suggested that the vessels of the skin of the forearm are under the control of the sympathetic nervous system, and that those of the muscle are not.

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BIBLIOGRAPHY

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4. Stead, E. A., Jr., Ebert, R. V., Romano, J., and Warren, J. V., Central autonomic paralysis. *Arch. Neurol. and Psychiat.*, 1942, 48, 92.
5. Lewis, T., and Grant, R. T., Observations upon reactive hyperemia in man. *Heart*, 1925, 12, 73.
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8. Kunkel, P., Stead, E. A., Jr., and Weiss, S., Blood flow and vasomotor reactions in the hand, forearm, foot, and calf in response to physical and chemical stimuli. *J. Clin. Invest.*, 1939, 18, 225.
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SENSITIVITY OF THE SMALLEST CUTANEOUS BLOOD VESSELS: QUANTITATIVE RESPONSES TO GRADED MECHANICAL STIMULATION AND TO LOCAL ISCHEMIA IN ARTERIAL HYPERTENSION, ARTERIOSCLEROSIS, AND CERTAIN ALLIED DISORDERS¹

BY JOSEPH R. DiPALMA AND FRANCES I. FOSTER

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(Received for publication May 11, 1942)

In hypertensive, as well as in normal, subjects, it is generally held that the level of arterial blood pressure is affected largely by the tone of the terminal arterioles; the vessels beyond are only passively concerned (1 to 4). There are, nevertheless, reports to the contrary (5, 6). These studies are, for the most part, based on direct and indirect mensuration of capillary pressure. There is considerable evidence, however, that the smallest blood vessels are involved functionally and morphologically in the disorders of the peripheral vascular tree, in ways other than that which may be demonstrated by the mere determination of the pressure relationship between capillaries and arterioles (7 to 10). The experiments reported here are designed to elucidate this problem by quantification of the responses of the smallest blood vessels in the skin of a selected group of patients, by the use of two methods recently described (11, 12). The first method measures the sensitivity of small dermal blood vessels to graded mechanical stroking (vasoconstriction), while the second measures the hyperemic response consequent to a standardized period of local ischemia (vasodilation). Individual, seasonal, segmental, and aging variations of these responses in normal subjects have been previously described (11 to 13).

METHODS AND PROCEDURES

The management of the patients employed in this study was as follows:² Procedures with any given clinic or ward patient were kept as standardized as possible. After

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² All patients used in these studies were selected from the medical wards and the cardiac clinic, Kings County Hospital, Long Island Medical College division, under the direction of Dr. J. Hamilton Crawford.

a review of the history, a careful physical examination was performed. Especial note was made of any abnormality pointing toward disease of the peripheral vessels. Every patient had a fundoscopic examination, an electrocardiogram, a six foot x-ray plate, blood chemistries, and several urinalyses.

The special determinations of small dermal blood vessel reactivity could usually be done following the physical examination. However, to maintain basal circulatory conditions, the patient rested quietly at least one hour before each determination. In every case, the skin of the ventral surface of the forearms was used as in our previous studies. A strength-duration curve was then determined using a mechanical device capable of varying both the speed and intensity of application of a stroker along the skin. This was done by finding the least weight in grams at each of five critical speeds of the stroker which produced a liminal degree of vasodilation against a background of vasoconstriction. The curve thus obtained was quantitated mathematically using the formula

recommended by Lassalle,³ $E = \frac{1}{\text{Rheobase}^2 \times \text{Chronaxie}}$.

(For full discussion of this method see (11).) Figure 1 illustrates samples of intensity-duration curves, obtained on three patients with different types of lesions.

The ability of small cutaneous blood vessels to respond by reactive hyperemia to local ischemia was then ascertained. This was done by application of a rubber ring, five square centimeters in area, to the skin of the forearm, the weight loading of which was 100 grams per square centimeter. By varying the length of time in seconds of application of the ring, an area of reactive hyperemia of even intensity, color, texture, and discreteness of edges could be obtained. This was called the *threshold*. By timing the period of time, in seconds, which such a hyperemic area required to fade to the color of the surrounding skin, the *clearing time* was obtained. (For full discussion of this method see (12).) The clearing time has been shown to be directly related to

³ In this investigation, only the Lassalle coefficient will be considered as the significant criterion of comparison of the intensity-duration curves. The rheobase, chronaxie, and the log-log slope of the curves were also analyzed, but it was found that they did not contribute to the clarity of the results.

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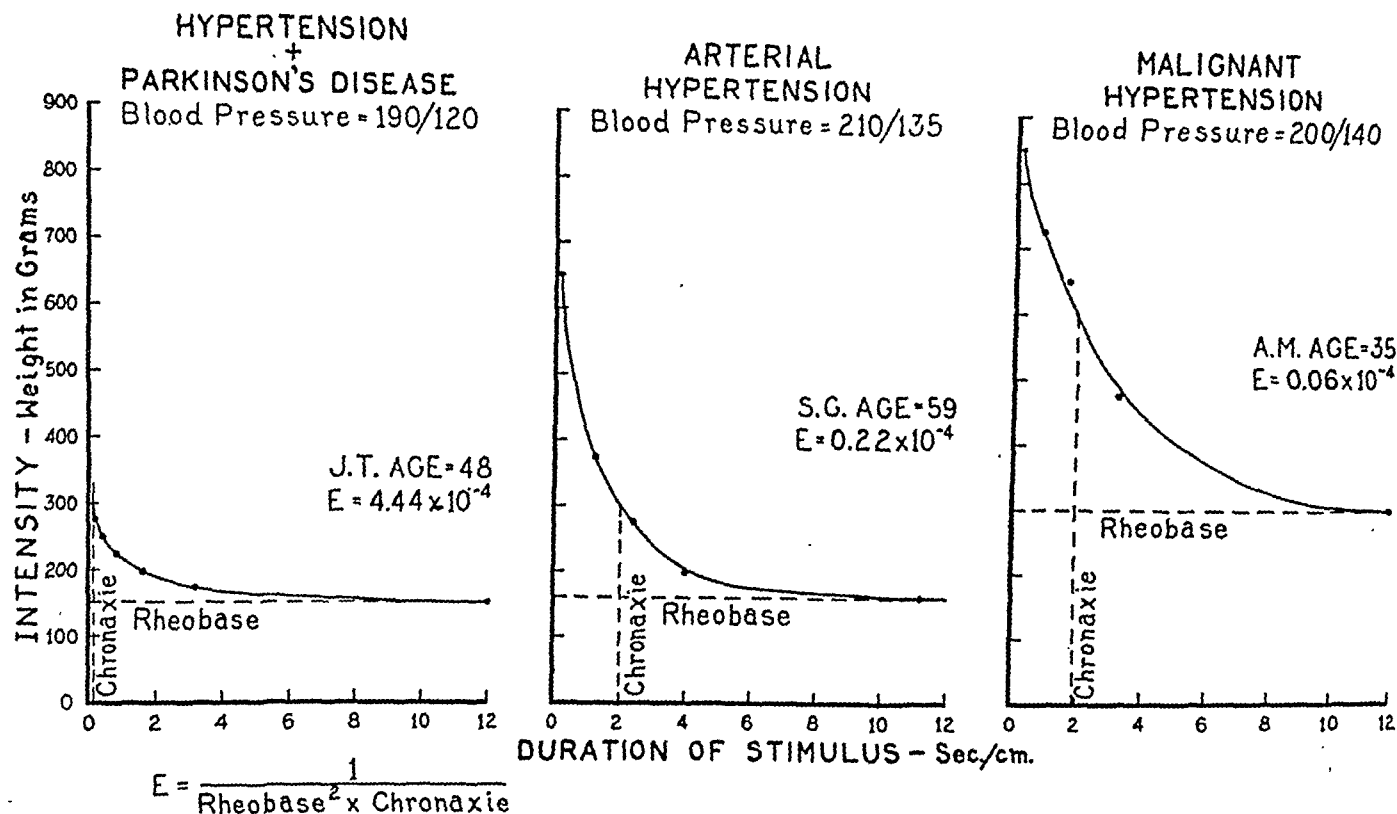


FIG. 1. METHOD OF PLOTTING THE INTENSITY-DURATION CURVES OBTAINED BY MECHANICAL STROKING OF THE SKIN

The Lassalle coefficient, E , is calculated as shown by graphically finding the values of the rheobase and the chronaxie. The three types of patients shown illustrate the range of capillary sensitivity found in this study.

the rate of cutaneous blood flow (12 to 14). Normal values will be discussed later.

No attempt was made to select an especial type of hypertensive patient. All those patients who came to our attention, in the cardiac clinic or on the medical wards, with a diagnosis of arterial hypertension or of arteriosclerosis were accepted for study. These, nat-

urally, included both early and late stages of arterial hypertension and of arteriosclerosis. The sex and age incidence of arterial hypertension of our group of patients agreed very well with that of other investigators (15). Most of the patients were in the sixth decade of life, with a spread of from the third to the eighth decades (see Table I).

TABLE I

Comparison of normal blood vessel reactions with those in arterial hypertension and in arteriosclerosis
Statistical analysis of the data obtained by graded mechanical stroking of the skin (capillary sensitivity).

Type of subject	Number in sample	Mean age	Reactive hyperemia*		Capillary sensitivity					
			Threshold, per cent of normal	Clearing time, per cent of normal	Mean coef. of excitability $\times 10^{-4} \dagger$	Standard deviation \pm	S. E. of the mean \pm	S. E. of difference from the normal mean \pm	t	Probability from Fisher's Table
Normal control	32	53	100	100	0.23	0.332	0.059			
Hypertensive	50	56	104	108	0.24	0.217	0.031	0.0667	0.1498	0.8-0.9
Hypertensive and arteriosclerotic	25	58	95	96	0.21	0.174	0.035	0.0686	0.2915	0.7-0.8
Arteriosclerotic	23	61	105	109	0.18	0.144	0.030	0.0654	0.7645	0.4-0.5
Malignant hypertensive	10	38	See Table II	See Table II	0.08	0.060	0.017	0.0614	2.443	0.02-0.05
Hypertension plus other lesions	13	55	See Table III	See Table III	1.73	1.087	0.301	0.306	4.902	Less than 0.001

* For discussion of the expected normal thresholds of reactive hyperemia in relation to age and season see text.

$\dagger E = \frac{1}{\text{Rheobase}^2 \times \text{Chronaxie}}$

Early in the investigation, it was found that some patients with arterial hypertension also had a considerable degree of arteriosclerosis, and *vice versa*. It was therefore deemed desirable to separate the group into three subgroups, *viz.*, hypertension, hypertension complicated by arteriosclerosis, and arteriosclerosis. The extent of arteriosclerosis was ascertained by the usual clinical methods, *i.e.*, history of coronary occlusion, intermittent claudication, etc., palpation of the radial and superficial temporal arteries, fundiscopic examination, and x-rays of the larger vessels.

For purposes of correlation with the capillary sensitivity test (graded mechanical stimulation), it was necessary to grade arbitrarily the severity of the disease process in the group of patients with uncomplicated arterial hypertension.⁴ This was done by following closely a plan similar to that advocated by Adson and Allen (17). Thus, by consideration of the general symptomatology,

⁴We feel that it is justifiable to refer to this test as a capillary sensitivity test. The term smallest blood vessels is more strictly proper, for the vessels affected undoubtedly include the finest arterioles and venules, besides the capillaries. However, Lewis (16) clearly presents the reasons why the white and red responses, upon which this test is based, may be considered an index of capillary reactivity.

changes in the retina, and the diastolic and systolic levels of blood pressure, it was possible to divide the patients into four main groups.

To obtain a basis of comparison, the same determinations were done upon a control group in the fifth and sixth decades of life, in which age group, most of our abnormal patients fell. These normals were selected from patients attending the eye clinic for refraction preparatory to the fitting of spectacles. A careful examination was done in each patient to exclude serious disease, especially that involving the cardio-vascular system and the skin.

RESULTS

Sensitivity to graded mechanical stimulation; capillary sensitivity. The results obtained in the normal group may be compared with those in the hypertensive group. By glancing at Table I, it will be seen that the mean coefficient of excitability for the normal group, 0.23×10^{-4} , is very similar to that of the hypertensive group, 0.24×10^{-4} . The coefficients for the hypertensive and arteriosclerotic groups also do not deviate markedly from that obtained in the normal.

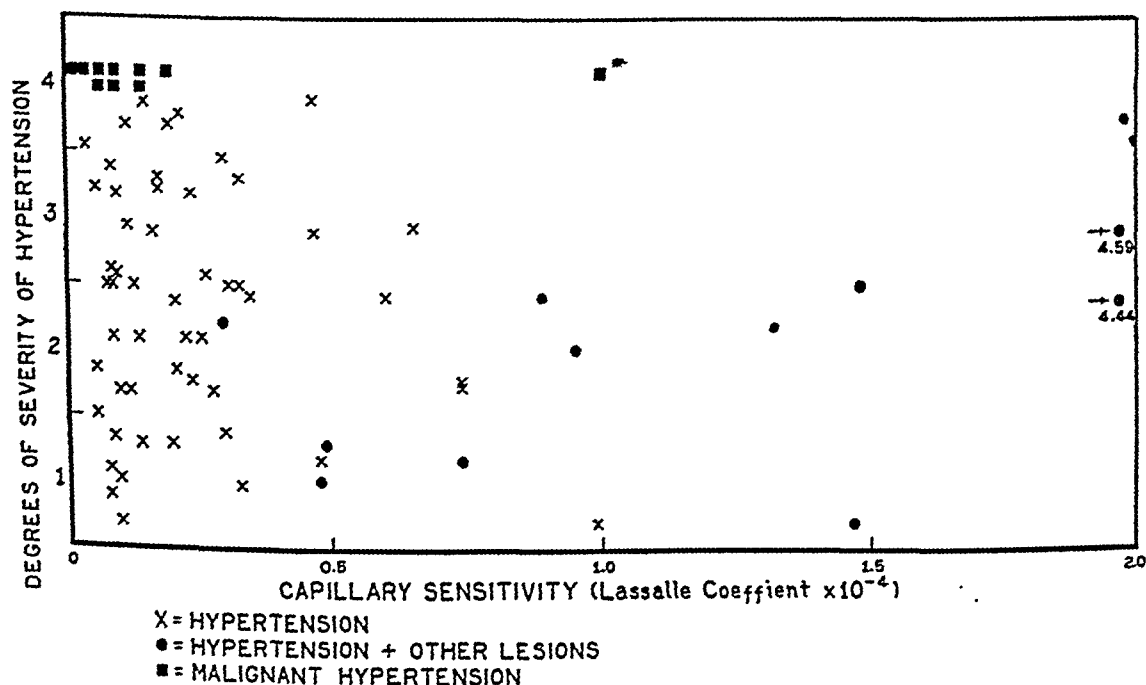


FIG. 2. THE LASSALLE COEFFICIENTS OF THE GROUP OF PATIENTS WITH ARTERIAL HYPERTENSION ARE PLOTTED AGAINST THE SEVERITY OF THEIR DISEASE AS DISCUSSED IN THE TEXT

Scattering of the crosses indicates the lack of correlation. Note that patients with malignant hypertension, and with hypertension associated with other lesions, fall into areas outside the general average of the uncomplicated hypertensives. The solid square marked by the asterisk is the coefficient of a patient who had malignant hypertension complicated by hemiplegia, with residual mental symptoms.

The results of the capillary sensitivity tests in the hypertensive group are graphically illustrated in Figure 2. The individual coefficients (in some cases, the average of as many as four determinations) are plotted against the severity of the hypertensive disease process, arbitrarily ascertained as described above in the section on methods. It is apparent from the scattering of the crosses that capillary sensitivity, as determined by mechanical stimulation of the skin, bears no relationship to the severity of the hypertensive lesions. A number of cases of malignant hypertension, and hypertension associated with certain other lesions, are also charted, which deviate widely from the uncomplicated hypertensive group. These will be discussed below.

Graphs were also plotted (not included in this paper) which permitted interpretation of the influence, if any, of the level of the diastolic and systolic pressure upon the individual coefficients. It was found that the level of either diastolic or systolic pressure had no correlation with the value obtained in the capillary sensitivity test. It is therefore probable that the level of the arterial blood pressure is not a significant factor in the sensitivity of the capillaries, as measured in this study. Furthermore, the capillary sensitivity is not influenced by the degree of severity of the hypertension, except as noted below. These conclusions also apply to the results obtained on patients with hypertension and arteriosclerosis, and with arteriosclerosis alone.

Responses to local ischemia; threshold of reactive hyperemia. The results of the reactive hyperemia test must be considered against the demonstrated normal seasonal variation in this reaction. In a previous study (12), on a large group of normal subjects, it was found that the threshold of reactive hyperemia varied from fifteen to twenty seconds in mid-summer, to seventy to eighty seconds in mid-winter. It was therefore necessary to collect seasonal data on normal subjects simultaneously with that obtained on the hypertensive and arteriosclerotic patients. This was done by weekly determinations on two normal subjects, throughout the course of this study. To this was added from time to time the normal data from other studies then in progress (13, 14). From a seasonal curve, thus constructed, the percentage deviation of the experimental data from

this normal data could be ascertained at any seasonal period throughout the year.

A threshold percentage greater than 100 per cent, or the expected seasonal normal, indicates less ability of the small dermal blood vessels to respond by reactive hyperemia to local ischemia. Similarly, a clearing time percentage greater than 100 per cent denotes a relatively slower cutaneous blood flow. The opposite relationship, obviously, also holds true. Since a scattering of as much as ten per cent may be present in a normal group, such a deviation cannot be considered abnormal in either group of data. It can be seen by glancing at Table I that the thresholds and clearing times of the reactive hyperemia tests do not deviate significantly from the normal, either in the hypertensive or arteriosclerotic groups. It is therefore evident that the small dermal blood vessels are not impaired in their capacity to respond to local ischemia, either in arterial hypertension or in arteriosclerosis. Furthermore, it is possible to infer, from a comparison of the clearing times, that the rate of blood flow in the skin also is not significantly different from the normal, since in previous studies it has been shown that the clearing time is directly related to the rate of blood flow in the skin (12 to 14).

Malignant hypertension. It must not be supposed, however, that the hypertensive process in its most severe phases will not ultimately influence the responses of the smallest dermal blood vessels. Eleven cases of the malignant phase of hypertension came to our attention, eight of whom had distinctly abnormal responses to these tests.⁵ These are summarized in Table II. With the exception of M. S., all had coefficients of excitability which were either normal or distinctly lower than normal. Thus, they ranged from 0.02, the lowest coefficient obtained in the entire series, to 0.20 which was that of the least ill member of the group, as judged by other standards. Patient M. S., who had a coefficient as high as 1.00 on one occasion and 0.38 about three months later, was the only one of the eleven who presented more sensitive dermal blood vessels than the average normal. However, she had a central nerve lesion which may markedly affect these blood vessel re-

⁵ Our viewpoints concerning the nature of malignant hypertension concur closely with those of Derow and Altschule (18).

sponses, as will be discussed later. It is therefore evident that the most severe phases of hypertension may be associated with a considerable decrease in the ability of the small dermal blood vessels to respond to mechanical stimulation.

The responses to local ischemia are even more abnormal. With the exception of R. S., five pa-

tients had a threshold of greater than 200 per cent of normal. Two patients had no reaction at all after five minutes stimulation time, and three patients had incomplete reactions at the end of five minutes stimulation. This is a distinctly abnormal finding, since no normal subject has ever been encountered on whom a response could not

TABLE II
Malignant hypertension

Patient	Sex	Age	Average blood pressure	Capillary sensitivity	Reactive hyperemia		Eye grounds	Urine†	Remarks
				Coefficient of excitability* ×10 ⁻⁴	Threshold, per cent of normal	Clearing time, per cent of normal			
R. S.	F	53	mm. Hg 230/140	0.20	147	100	Exudate; hemorrhage; blurred discs	S.G. = 1021 Alb. = 3+	Excellent skin color
N. C.	F	47	220/110	0.04	300	230	Exudate; hemorrhage	S.G. = 1023 Alb. = 2+	Pale, pigmented skin color
M. S.	F	52	268/180	1.00 0.38	No reaction after 5 minutes		Exudate; extreme spasm of vessels	S.G. = 1026 Alb. = 1+	Hemiplegia with residual paralysis and extreme mental symptoms. Very pale, pigmented skin color
M. V.	F	30	232/156	0.08 0.11	Not complete at 5 minutes		Papilledema; old and fresh hemorrhages	S.G. = 1015 Alb. = 1+	Very pale skin
B. B.	F	52	230/155	0.02	273	137	Exudate; hemorrhage; edema of retina	S.G. = 1015 Alb. = 3+ 4+	Pale, pigmented skin color
M. D.	F	33	260/158	0.05	253	442	Patchy exudate; fresh hemorrhages	S.G. = 1016 Alb. = 1+	Pale, pigmented skin color (Negroid)
A. M.	M	35	200/140	0.06	650	320	Exudate; hemorrhage; blurred discs	S.G. = 1015 Alb. = 2+ 3+	Pale, pigmented skin color
G. P.	M	45	210/132	0.10	No reaction after 10 minutes		Papilledema; old and fresh hemorrhages	S.G. = 1008 Alb. = 2+ 3+	Pale, pigmented skin color
J. W.	M	48	214/150	0.07	303	213	Marked, soft exudate; hemorrhage; spastic closed arteries	S.G. = 1010 Alb. = 2+ 4+	Pale, pigmented skin color. Died 4 days later in uremia
M. L.	M	23	264/148	0.16	Not complete at 5 minutes		Papilledema	S.G. = 1012 Alb. = 4+	Pale, pigmented skin color
M. D.	M	33	210/120	0.15	Not complete at 5 minutes		Blurred discs	S.G. = 1012 Alb. = 2+	Moderately pale skin color

* $E = \frac{1}{\text{Rheobase} \times \text{Chronaxie}}$

† All had abnormal quantities of formed elements in their urine.

TABLE III
Hypertension associated with certain other lesions

Patient	Sex	Age	Average blood pressure	Capillary sensitivity	Reactive hyperemia		Remarks
				Coefficient of excitability $\times 10^{-4} \dagger$	Threshold, per cent of normal	Clearing time, per cent of normal	
A. E.....	F	56	mm. Hg 185/122	1.48	No reaction after 5 minutes		Hemiplegia with residual paralysis and mental symptoms
R. W.....	F	54	146/84	1.47	73	88	Neurasthenia
J. V.....	F	63	140/90	4.59	83	80	3 years ago had high B. P.; after heart attack, had low B. P. Headaches
E. O.....	F	35	140/100	0.95	94	117	Post-encephalitic.
L. A.....	F	68	130/90	0.48	42	45	Parkinson's syndrome
M. M.....	F	39	130/85	0.49	85	81	Parkinson's disease.
M. C.....	F	73	135/85	0.74	49	56	Paralysis agitans
C. W.....	F	50	160/100	0.30	73	56	Post-encephalitic.
J. D.....	M	58	185/120	1.98	170	53	Parkinson's syndrome
J. T.....	M	48	190/120	4.44	93	60	Parkinson's disease.
W. K.....	M	77	220/120	1.32	83	120	Paralysis agitans
M. S.....	M	31	140/100	0.89	77	71	Parkinson's disease.
N. M.....	M	49	164/110	2.13	48	141	Paralysis agitans
							Post-encephalitic.
							Parkinson's syndrome
							Post-encephalitic.
							Parkinson's syndrome

$$\dagger E = \frac{1}{\text{Rheobase}^2 \times \text{Chronaxie}}$$

be elicited in ninety seconds with the application of local ischemia, even at the height of the seasonal curve (12). The clearing times in those patients on whom a response could be elicited were as much as 200 per cent greater than normal clearing times, denoting a slowing of cutaneous blood flow. These results, therefore, disclose a marked impairment in the ability of the smallest cutaneous vessels of patients in the malignant phase of hypertension to respond by reactive hyperemia to local ischemia.

The clinical finding of a pale and often pigmented skin in these patients substantiates the dermal blood vessel tests. It is notable that the one patient in the group, R. S., who had normal skin, also had practically normal cutaneous blood vessel reactions (Table II).

Hypertension associated with certain other lesions. Thirteen patients with various types of

nerve lesions, ranging from hemiplegia with residual symptoms to Parkinson's disease, who had abnormal cutaneous blood vessel reactions, were also studied. Thus patient J. T., Table III and Figure 1, had a coefficient of excitability to mechanical stimulation of 4.44, a figure nearly eighteen times that of the mean average for the corresponding group of hypertensives. All the others had coefficients far greater than normal (Table III).

The responses to local ischemia in this group, with the exception of patient A. E. (Table III), substantiate the results of the mechanical stimulation test. Throughout this series, the threshold and clearing times were shorter than the expected normal. This denotes cutaneous vessels more sensitive to local ischemia, and a faster dermal blood flow than in normal skin.

In the light of these findings of increased small dermal blood vessel sensitivity in hypertension with certain associated disorders and especially in central nerve lesions, the high coefficient of case M. S., Table II, a malignant hypertensive, can now be explained. One year previous to admission she had a stroke which resulted in complete hemiplegia, speech defects, and marked disorientation. This, undoubtedly, lead to a coefficient of 1.00 despite her advanced stage of hypertension.

Various other diseases, and especially central nerve lesions, such as hemiplegia and Parkinson's syndrome, greatly increase the responsiveness of small cutaneous blood vessels, both to mechanical stimulation and to local ischemia, even in the face of advanced hypertension. The reasons for this cannot be elucidated in this investigation, however. Further study of this type of patient is necessary.

Statistical treatment. For purposes of analysis, the data were originally separated into male and female groups. No significant differences could be obtained, however, by various tests between these groups. Therefore, the data were combined for presentation in this communication. Moreover, since there were nearly an equal number of males and females in each main group, any differences which might be present neutralized each other.

In order to ascertain further just what significance might be laid on the differences between the mean of the capillary sensitivity tests on the normals as opposed to the experimental groups, the data were treated statistically following the recommendations of Mainland (19). Since, in data of this type, the probability (Fisher's Tables (20)) must be at least 0.05 or less to be significant, it may be seen in Table I that the conclusions which have been placed on the data thus far are correct. Thus the data of the hypertensive, hypertensive and arteriosclerotic, and the arteriosclerotic groups have probabilities of 0.8, 0.7, and 0.4 respectively, putting these data far out of the realm of significance. The malignant hypertensive group has a probability of 0.02 to 0.05, which is just significant. Doubtless, if more of this type of patient could have been studied, the significance would have been greater. The group of hypertensives with associated lesions has a probability of less than 0.001 which may be regarded as highly significant. Therefore one is assured that the dif-

ferences found in the capillary sensitivity tests were real rather than coincidental.

DISCUSSION

It is not surprising to find that the functional responses of the smallest cutaneous blood vessels in various degrees of arterial hypertension, and in arteriosclerosis, are not different from those in normal skin. This is entirely consonant with the viewpoints of others on the dynamics of the circulation in arterial hypertension (1 to 7). The clinical observation that the skin of hypertensive patients shows no signs of atrophy, and presents variations of coloring and texture no different from the skin of normals in the same age range, further substantiates these findings. The observation that the capillaries in the nail fold of arteriosclerotics are often "moth eaten" (21), as seen by use of the capillary microscope, in no way vitiates the conclusion that the small blood vessels of the skin in these patients are not impaired in their functional capacity to respond to mechanical stimulation and to ischemia. There are, no doubt, morphological changes in the smallest vessels with advancing vascular disease, but this does not necessarily imply that their functional reserve to respond to local injury is exceeded (14).

The disclosure of distinctly decreased irritability and impairment of the capacity to respond to local ischemia of the small dermal vessels in the malignant phase of hypertension deserves further consideration. It may be advanced that the extreme narrowing of the arterioles in this condition results in actual obliteration of the vessels beyond, and that this, in reality, causes the appearance of lessened irritability.

There are reasons why this view is not tenable. First, if there is complete obliteration of the capillaries in a certain area, complete disappearance of the tissue supplied by these vessels must result, for cellular life is dependent on capillary blood exchange (21). Such is not observed to be the case in the skin of patients with the malignant syndrome of hypertension, however. Second, it has been shown on a large group of patients, with extreme degrees of organic obliterative vascular disease not complicated by hypertension, that the small dermal vessels do not lose, in any way, their ability to respond by reactive hyperemia to local

ischemia (14). Third, a patient has been observed who, despite an advanced stage of hypertension, had unusually sensitive small cutaneous blood vessels (case M. S., Table II).

Another probable supposition is that the agent which causes constriction of arterioles in the malignant phases of hypertension extends its influence to the smaller vessels beyond. A finding which lends some support to this viewpoint is the recent observation of Wilkins and Duncan (9) that angiotonin, injected intracutaneously, causes local blanching, although it is not as marked as that caused by a similar injection of epinephrine. In this regard, we have not only been able to confirm this finding, but to extend it to our studies. By iontophoresis of a small amount of epinephrine into the skin of a normal subject, it is possible to change the responses of the small dermal vessels, as measured in this paper, so as to resemble closely those obtained in the malignant phase of hypertension. That is, the capillary sensitivity decreases greatly and the response to local ischemia is abolished. An attempt was made to duplicate this result with angiotonin, with only partial success. This was due to the difficulty of introducing angiotonin into the skin by iontophoresis.⁶

Volhard's clinical separation of hypertensive patients into those with red and those with pale skin may have more merit to it than it has been commonly accorded (22). His conception, however, that the color of the skin is mainly dependent upon the caliber of the arterioles is erroneous, as has been properly pointed out by Fishberg (7). On the other hand, his impression that in the later stages of hypertension there appears in the blood stream a vaso-pressor substance which causes universal vaso-constriction finds some support in our studies. Thus, in the early stages of hypertension, the quantitated responses of the small vessels of the skin are no different from those in normal skin, whereas in the malignant phases of the disease they show a marked decrease in sensitivity, indicating an increased tone. Since it is the smaller blood vessels of the skin which determine its color (16), it may be seen that the increases in the tone of these vessels may result in the pale skin of the late phases of hypertension,

observed by Volhard. Just what relationship the degree of renal damage, held to be responsible for these changes by Volhard, has to the responses of the small dermal vessels remains to be ascertained.

These findings of abnormal small cutaneous vessel responses in the extreme stages of hypertension, if confirmed and extended, might supply a reliable criterion of the extent of the vascular lesion in this condition. It might contribute to the answer of the problem of just when an apparently benign hypertension is converted to a malignant phase. The rapidly fatal course of this disease may, in itself, result from eventual involvement of the smallest blood vessels, with all that implies.

SUMMARY AND CONCLUSIONS

The responses to graded mechanical stimulation, and to local ischemia of the smallest blood vessels of the skin of the ventral surface of the forearm, were quantitated in fifty patients with arterial hypertension, twenty-five patients with arterial hypertension associated with arteriosclerosis, and twenty-three patients with arteriosclerosis. Also included in this study were eleven cases of malignant hypertension, and thirteen cases of hypertension associated with various types of nerve lesions, which influenced their capillary sensitivity. These results were compared to similar studies on a suitable control group of thirty-two subjects. The implications of the abnormal responses obtained were discussed. The following conclusions were reached.

1. In the group with arterial hypertension, it was demonstrated that the responses of the small dermal vessels, as quantitated in this study, are in no way significantly different from those of a comparable normal group.

2. No relationship was found between the severity of the hypertensive process, excluding the malignant phase, and the functional responses of the small cutaneous vessels. Many cases of very severe hypertension, with diastolic blood pressures of over 130 mm. of Hg, were studied, and showed normal capillary responses.

3. The conclusions for the purely hypertensive group apply as well to those patients with hyper-

⁶ Unpublished observations. Angiotonin was generously supplied to us by Dr. Irvine H. Page of the Lilly Medical Research Laboratories.

tension associated with arteriosclerosis, and with uncomplicated arteriosclerosis.

4. Of eleven patients with the malignant syndrome of hypertension, ten had small blood vessel responses which indicated greatly decreased sensitivity. This was especially evidenced, in five of these patients, by a complete inability of the small dermal vessels to respond by reactive hyperemia to local ischemia.

5. Thirteen patients with hypertension complicated by a nerve lesion, ranging from a cerebral vascular accident to Parkinson's disease, were found to have small cutaneous vessels as much as eighteen times more sensitive than the normal or hypertensive groups. Very irritable, small, dermal blood vessels may therefore exist even in the presence of arterial hypertension.

6. The above conclusions suggest that the humoral agent now believed responsible for arterial hypertension does not exert its influence upon the smallest blood vessels in the benign stages of the disease but may do so in the later malignant phase. If this is confirmed, the quantitative responses of the small dermal blood vessels might serve as a criterion of the extent of the vascular lesions in advancing hypertensive disease.

BIBLIOGRAPHY

1. Ellis, L. B., and Weiss, S., The measurement of capillary pressure under natural conditions and after arteriolar dilatation: In normal subjects and in patients with arterial hypertension and with arteriosclerosis. *J. Clin. Invest.*, 1929, 8, 47; and also, *Am. Heart J.*, 1930, 5, 448.
2. Wiggers, C. J., The dynamics of hypertension. *Am. Heart J.*, 1938, 16, 515.
3. Griffith, J. Q., Jr., Roberts, E., and Corbit, H. O., Studies of criteria for classification of arterial hypertension. I. Cutaneous capillaries. *Am. Heart J.*, 1941, 21, 47. II. Minute vessel pressure. *Ibid.*, 1941, 21, 54.
4. Eichna, L. W., and Bordley, J., III, Capillary blood pressure in man. Direct determination in the digits of subjects with normal and elevated arterial pressures. *J. Clin. Invest.*, 1941, 20, 458.
5. Boas, K., and Mufson, I., The capillary blood pressure in arterial hypertension and in nephritis. *J. Lab. and Clin. Med.*, 1923, 9, 152.
6. Mufson, I., A study of the capillary pressure in nephritis and hypertension. *Am. J. M. Sc.*, 1932, 183, 632.
7. Fishberg, A. M., Hypertension and Nephritis. Lea and Febiger, Philadelphia, 1931, 2nd Ed.
8. Cowdry, E. V., Editor, Problems of Ageing. Biological and Medical Aspects. Williams and Wilkins, Baltimore, 1939.
9. Wilkins, R. W., and Duncan, C. N., The nature of the arterial hypertension produced in normal subjects by the administration of angiotonin. *J. Clin. Invest.*, 1941, 20, 721.
10. Griffith, J. Q., Jr., The frequent occurrence of abnormal cutaneous capillaries in constitutional neurosthenic states. *Am. J. M. Sc.*, 1932, 183, 180.
11. DiPalma, J. R., Reynolds, S. R. M., and Foster, F. I., Sensitivity of the smallest blood vessels of the human skin: Responses to graded mechanical stimulation in normal males. *J. Clin. Invest.*, 1941, 20, 333.
12. DiPalma, J. R., Reynolds, S. R. M., and Foster, F. I., Quantitative measurement of reactive hyperemia in human skin: Individual and seasonal variations. *Am. Heart J.*, 1942, 23, 377.
13. DiPalma, J. R., and Foster, F. I., The segmental and ageing variations of reactive hyperemia in human skin. *Am. Heart J.*, 1942, 24, 332.
14. DiPalma, J. R., and Foster, F. I., A reactive hyperemia ring test in the study, evaluation and prognosis of pedal lesions caused by obliterative vascular disorders. *Am. Heart J.*, 1942, 24, 345.
15. Riseman, J. E. F., and Weiss, S., The age and sex incidence of arterial hypertension. *Am. Heart J.*, 1930, 5, 172.
16. Lewis, T., The Blood Vessels of the Human Skin and Their Responses. Shaw, London, 1927.
17. Adson, A. W., and Allen, E. V., Essential hypertension. General considerations. *Proc. Staff Meet., Mayo Clin.*, 1937, 12, 1.
18. Derow, H. A., and Altschule, M. D., The nature of malignant hypertension. *Ann. Int. Med.*, 1941, 14, 1768.
19. Mainland, D., The Treatment of Clinical and Laboratory Data. Oliver and Boyd, Edinburgh, 1938.
20. Fisher, R. A., and Yates, F., Statistical Tables for Biological, Agricultural and Medical Research. Oliver and Boyd, Edinburgh, 1938.
21. Weiss, S., and Frazier, W. R., The density of the surface capillary bed in the forearm in health, arterial hypertension and in arteriosclerosis. *Am. Heart J.*, 1930, 5, 511.
22. von Volhard, F., Der arterielle Hochdruck. Verhandlungen der Deutschen Gesellschaft für Innere Medizin. Verlag von J. F. Bergmann, München, 1923.

THE EFFECTS ON RENAL RESISTANCE TO BLOOD FLOW OF RENIN, ANGIOTONIN, PITRESSIN AND ATROPINE, HYPERTENSION, AND TOXEMIA OF PREGNANCY¹

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The interpretation of the observations on the clearances of inulin and diodrast, as measures of rate of glomerular filtration and of renal plasma flow, has been handicapped by lack of quantitative criteria of changes in afferent and efferent renal arteriolar resistance. Most of the qualitative criteria suffer because they do not take account of simultaneous changes in blood pressure, and because they over-simplify by ascribing the effect either to the afferent or the efferent arterioles alone. Where so many factors are interrelated, as in the components determining renal arteriolar resistance, mathematical relationships are necessary. We have previously offered formulas for renal arteriolar resistance (1, 2). They have here been applied to data on renal function in dogs, and also in man, published by Corcoran and Page *et al.* (3 to 7), whom we wish to thank for their kindness in putting at our disposal supplemental and unreported observations.³ We are also grateful to Mr. Julius Stein for his painstaking aid in computation.

METHOD

The formulas for afferent and efferent renal arteriolar resistance are respectively:

$$R_A = \frac{P_M - P_{O'} - 40}{HD};$$

$$R_E = \frac{(1 - 0.47F)(P_{O'} - P_O + 10)}{HD};$$

$$P_{O'} = \frac{2.34S}{1 - 0.0542S - F}.$$

Here P_M is the mean of systolic and diastolic blood pressure; P_O is the osmotic pressure of the systemic blood ($F = 0$), while $P_{O'}$ is the osmotic pressure of the blood after a fraction, F , of the plasma has been filtered off in the glomerulus. These pressures are measured in millimeters of mercury. D is the diodrast clearance or effective renal plasma flow in cc. per minute per individual, or per unit surface area. No allowance is made for discrepancy between D and effective plasma flow. I is the inulin clearance in the same units as D . $F = I/D$. S is the concentration of serum protein in grams per 100 cc. No correction for $A : G$ ratios differing from our standard of 2.20 has been applied, but means for such adjustment are available where the ratios are known (2). H is the reciprocal of 1-hematocrit, expressed as a fraction of 1, and HD is therefore effective renal blood flow. Since Corcoran and Page in their animal experiments found the rate of renal blood flow in the exteriorized kidney by the Fick principle, analyzing arterial and renal venous blood directly for both phenol red and inulin, we use the average of their plasma flow values derived from these two substances for our D . P_O , glomerular intra-capillary pressure, is given by the approximation, $P_{O'} + 20$ (1).

The sum of arteriolar resistance is $R = R_A + R_E$. Being resistances, each of these symbols is properly expressed in terms of the units of the formulas: millimeters of mercury per cc. individual's own blood per minute per individual, or per unit surface area. In all cases, except those with toxemia of pregnancy, the resistance is referred to the individual's own blood as standard perfusing medium, so that the resistance values for one individual are not quite comparable with those of another.

In many of the experiments reported, there is more than one control or experimental period. Strictly speaking, the resistance of the afferent and efferent arterioles for each observation should be calculated separately, and averaging of the whole set of final resistances should be done. However, in a trial series, we studied this method as compared to averaging the individual quantities from which a single resistance is drawn and found no statistically significant difference. Consequently, we have adopted this simpler method of computation, except in a few instances where wide variability within the periods to be averaged suggested individual calculations. For the sake of completeness, we restate the results of Corcoran and Page for blood pressure, renal blood flow, and glomerular filtration rate; the values for glomerular intra-capillary pressure and for arteriolar resistance and its related quantities are original.

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³ The supplemental data were the hematocrit values which are here supplied in full in the form of $1/(1\text{-hematocrit}) = H$. All unreported cases are indicated as such in the tables, where all the data for calculation are supplied. We are assured by the authors that the technique and conditions of the unreported cases were the same as those already reported, with which they are included.

The precision of measurement of renal arteriolar resistance is touched on elsewhere (1,2). It is, of course, clear that results drawn from a formula depend on the accuracy of the data applied. The measurements of clearance are subject to an error estimated at about 10 per cent. In evaluating results, the quality and mass of the data must be considered. We shall not report conclusive changes in numerical quantities, since here this does not seem warranted. However, while it may be incorrect to say, for example, that a 42 per cent increase in a particular quantity was noted, it may be statistically legitimate to report in such an instance simply that an increase occurred.

RESULTS

1. *Effect of pitressin*

Eight experiments were performed on unanesthetized dogs after the initial results of pitressin had worn off (3). No consistent change in glomerular intra-capillary pressure occurred, nor do changes appear correlated to the degree of blood pressure shift, which is mainly that of slight, usually inconsequential, increase. Total renal arteriolar resistance, while it changed considerably, was not consistent in direction. There is also no consistent change in the ratio of afferent to efferent arteriolar resistance; half of the cases showed an increase and the other half a decrease. The suggestion that constriction of the efferent arterioles occurs during periods of reduced renal blood flow is not supported by the calculations. No particular anatomical site of action or functional effect of pitressin is revealed (Table I).

2. *Effect of atropine after pitressin*

While the addition of intravenous atropine to pitressin, as previously reported (3), did not consistently affect renal blood flow, blood pressure was markedly elevated, and glomerular filtration rate was increased. Despite the marked blood pressure increase, glomerular capillary pressure rose considerably in only 2 of the 5 cases, out of a total of 7, in which an increase was recorded, and in these 2 cases the blood pressure shift was minor. Statistical analysis reveals that the increase is not significant (23 per cent likelihood of chance alone being responsible). In all cases, total arteriolar resistance rose, with afferent arteriolar constriction predominating in all but 1 case. In fact, the efferent arterioles dilated in 4 of the 7 cases (Table I).

3. *Effect of renin*

Aside from the increase in blood pressure, and fall in renal blood flow (3), renin consistently increased renal arteriolar resistance but, while the increase was predominantly afferent 6 times, it was mainly efferent in origin 8 times. There was a fairly consistent rise in glomerular intracapillary pressure, and an inconsistent tendency to fall in glomerular filtration rate (Table II).

4. *Effect of angiotonin in dogs*

We are indebted to Corcoran and Page for permitting the use of their published experiments with angiotonin (4), and for supplying data supplemental to these published reports. From them have been selected those cases in which calculation showed the glomerular intracapillary pressure to be less than blood pressure. It is possible that our formula for glomerular intracapillary pressure, which depends on the Adairs' and Greaves' observations on osmotic pressure of diluted serum (8), is incorrect at high degrees of hemoconcentration. There are technical difficulties, too, in the measurement of renal blood flow and inulin clearance during the rapid fluctuations produced by angiotonin. For these reasons, as well as the possibility that the formulas may not apply at very high degrees of vasoconstriction (2), the results for angiotonin are subject to doubt. Their similarity to those obtained with renin suggests, however, that they are probably not too unreliable for a first estimate of the effect on renal resistance of angiotonin, particularly in view of the current interest in this drug and the, as yet, unsupported opinions of its mode of activity.

Renal blood flow fell, blood pressure rose, and renal resistance increased markedly during angiotonin infusion in the cases selected as noted. The increased resistance cannot here be ascribed primarily to afferent or efferent arteriolar constriction. On the whole, glomerular intracapillary pressure rose. Changes in glomerular filtration rate were not consistent (Table III).

5. *Effect of angiotonin in man*

Figure 1 shows the previously reported results of angiotonin infusion in a single human subject (5). The calculations of total arteriolar resist-

TABLE I
Effect of pitressin and pitressin with atropine in dogs

Dog	Drug	Glomerular filtration rate f_g		Blood flow HD		Filtration fraction $f_g = f_D$	Blood pressure P_M		Glomerular intracapillary pressure P_G		Afferent arteriolar resistance R_A		Efferent arteriolar resistance R_E		Total arteriolar resistance R		Afferent resistance Efferent resistance R_A/R_E
		cc. per square meter per minute	per cent	cc. per square meter per minute	per cent		mm. Hg	per cent	mm. Hg	per cent	mm. Hg per liter per square meter per minute	per cent	mm. Hg per liter per square meter per minute	per cent	mm. Hg per liter per square meter per minute	per cent	
249	Control Pit. Pit. At.	62.0	+40 +44	291	+14 +31	38.0 43.4 37.8	146 +4 +37	85.7 +21 -1	138	-39 +80	139	+16 -24	278	-12 +28			1.00 0.52 2.37
249*	Control Pit. Pit. At.	73.3	+4 +12	535	-19 -41	26.8 32.0 46.7	127 +3 +42	65.4 +11 +85	77.8	+13 +65	47.3	+51 +342	125	+27 +170			1.64 1.23 0.61
250	Control Pit. Pit. At.	68.5	+5 +27	435	-14 -8	32.8 37.0 41.5	146 +2 +36	76.1 +12 +32	115	+2 +71	77.2	+41 +65	192	+18 +69			1.48 1.07 1.54
248	Control Pit. Pit. At.	84.2	+16 +20	511	+44 -6	30.5 21.4 32.9	120 +13 +90	72.1 -16 +6	54.6	+38 +402	59.8	-51 +18	114	-8 +201			0.91 2.58 3.90
244	Control Pit.	82.2	-6	505	-14	28.5 28.2	123 +18	69.0 -1	67.3	+93	55.9	+15	123	+58			1.20 2.03
289	Control Pit. Pit. At.	63.5	+12 +46	325	+66 +31	34.3 21.7 35.1	129 +5 +44	79.2 -23 +2	91.7	+12 +117	111	-63 -22	203	-29 +41			0.83 2.54 2.29
329	Control Pit. Pit. At.	77.1	+11 +36	481	+22 +27	30.8 28.6 32.8	149 -13 +24	72.6 -5 +5	117	-40 +22	64.4	-25 -15	182	-35 +9			1.82 1.44 2.61
301	Control Pit. Pit. At.	63.4	+34 +47	427	+25 +45	28.6 27.4 25.0	142 +4 +28	69.0 -2 -7	124	-8 +26	66.2	-23 -40	190	-14 +3			1.87 2.24 3.91

* Another experiment on the same animal but on a different day.

TABLE 11.
Effect of renin in dogs

TABLE 11 Effect of renin in dogs																		
Dog	Drug	Glomerular filtration rate		Blood flow		Filtration fraction $F = I/D$	Blood pressure		Glomerular intra-capillary pressure		Afferent arteriolar resistance		Efferent arteriolar resistance		Total arteriolar resistance		Afferent resistance R_A/R_E	
		cc. per square meter per minute	per cent	cc. per square meter per minute	per cent		mm. Hg	per cent	mm. Hg	per cent	mm. Hg per liter per square meter per minute	per cent	mm. Hg per liter per square meter per minute	per cent	mm. Hg per liter per square meter per minute	per cent	mm. Hg per liter per square meter per minute	per cent
249	Control Renin	57.9	0	402	-29	27.2	132	+10	65.8	+44	115	- 7	63.8	+158	179	+ 52	1.80	0.65
249*	Control Renin	66.0	+14	377	- 2	41.0	133	+19	74.7	+ 5	102	+ 57	85.7	+ 10	188	+ 36	1.19	1.69
250	Control Renin	78.9	-17	546	-51	33.0	123	+20	67.9	+48	64	+ 59	50	+286	114	+159	1.28	0.53
248	Control Renin	67.5	-21	393	-23	35.0	123	+18	70.6	+ 9	82	+ 94	75.0	+ 52	157	+ 74	1.10	1.40
248*	Control Renin	67.5	+ 3	386	-25	29.6	124	+22	72.4	+34	82.0	+ 43	79.7	+112	162	+ 76	1.03	0.69
244	Control Renin	68.0	-28	382	-27	30.7	132	+38	75.4	- 8	95.9	+245	60	+208	147	+114	1.11	3.26
244*	Control Renin	74.8	+12	465	-35	40.7	129	+29	68.6	+56	87.0	+ 49	67.8	+178	118	+164	1.45	0.70
289	Control Renin	74.7	- 1	416	-35	28.2	110	+39	69.0	+45	50.6	+140	60.6	+330	124	+230	0.75	0.64
329	Control Renin	85.2	-24	496	-51	43.1	123	+40	71.4	+63	63.6	+132	50.9	- 19	130	+ 23	1.05	0.57
329*	Control Renin	79.3	- 7	561	+ 6	28.5	134	+16	69.5	- 8	79.4	+ 50	55.9	+351	167	+113	1.56	2.89
329*	Control Renin	67.8	+ 2	484	-42	41.5	141	+26	67.5	+90	111	- 7	50	+328	235	+102	1.98	0.41
301	Control Renin	41.4	+ 7	414	-48	27.4	156	+ 8	59.4	+57	185	+ 40	99.3	+105	216	+127	3.68	1.21
239	Control Renin	48.6	-15	304	-36	20.4	127	+26	71.6	+17	117	+145	117	+105	183	+131	1.17	1.42
320	Control Renin	53.0	- 6	283	-26	39.6	114	+37	75.3	+29	66.3	+184	117	+100	183	+131	0.57	0.80

other experiment on the same animal but on a different day.

* Another experiment on the same animal but on a different day.

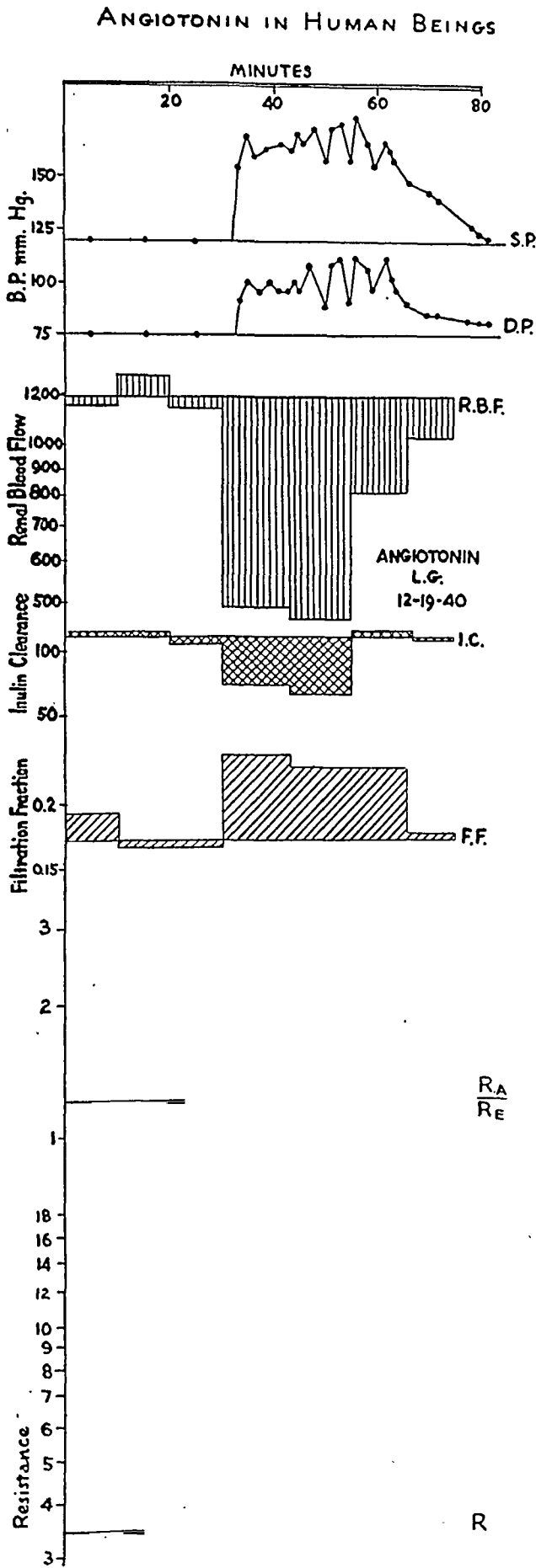
TABLE III

Effect of angiotonin in dogs

Dog	Drug	Glomerular filtration rate I		Blood flow HD		Filtration fraction $F = I/D$	Blood pressure P_M		Glomerular intra-capillary pressure P_G		Afferent arteriolar resistance R_A		Efferent arteriolar resistance R_E		Total arteriolar resistance R		Afferent resistance Efferent resistance R_A/R_E
		cc. per square meter per minute	per cent	cc. per square meter per minute	per cent		mm. Hg	per cent	mm. Hg	per cent	mm. Hg per liter per square meter per minute	per cent	mm. Hg per liter per square meter per minute	per cent	mm. Hg per liter per square meter per minute	per cent	
575	Control Angiot.	33.1	+ 3	187	-25	29.6 39.5	112	+32	71	+31	115	+116	158	+108	273	+111	0.73 0.76
574	Control Angiot. Angiot.	59	-42 -56	344	-55 -55	27.2 34.0 26.3	122	+16 +14	67	+18 - 1	101	+174 +239	78	+192 +115	179	+182 +185	1.30 1.23 2.06
574*	Control Angiot.	69.7	-40	346	-61	29.7 44.3	120	+36	71	+59	85	+159	85	+595	170	+345	0.99 0.50
572†	Control Angiot. Angiot.	58	+ 3 0	476	- 1 + 4	29.0 27.8 25.7	130	+15 +11	70	- 3 - 7	85	+ 54 + 41	61	- 3 - 15	145	+ 30 + 18	1.40 2.22 2.33
596†	Control Angiot. Angiot. Angiot.	45	+22 + 9 - 4	350	-14 -19 -25	25.5 34.0 31.9 30.1	123	+ 9 +15 +11	65	+22 +14 + 9	109	+ 9 + 54 + 56	72	+ 64 + 59 + 59	181	+ 31 + 56 + 57	1.51 1.01 1.47 1.48

* Another experiment on the same animal but on a different day.

† Unreported experiments by Corcoran and Page performed after publication of the others (4), but otherwise similar.



ance, R , and the afferent-to-efferent arteriolar ratio, R_A/R_E , are original. This case is of especial interest, partly because man is the subject, partly because it illustrates the value of a quantitative estimate of renal arteriolar resistance. Aside from the changes shown, which correspond to those in the dog, there was moderate increase in glomerular intra-capillary blood pressure. The rise in filtration fraction (FF) with the fall in blood flow (RBF) had been considered "the characteristic result of constriction of the glomerular efferent arterioles." It is seen, however, from R_A/R_E that afferent arteriolar resistance increased proportionately more than efferent arteriolar resistance (Figure 1).

6. Ratio of afferent to efferent arteriolar resistance in human hypertension

The data in Table IV are calculated from those reported in essential hypertension by Goldring

TABLE IV
Afferent-to-efferent arteriolar ratio in essential hypertension

Mean blood pressure mm. Hg.	Afferent resistance Efferent resistance
124	1.58
122	1.46
158	2.36
156	3.67
152	2.56
163	2.76
129	1.65
162	2.88
139	2.72
165	3.06
160	3.53
195	5.61
173	2.46
122	2.48
137	2.58
155	3.85
162	2.66
Average	2.82
Normal ♂ 100	1.13
Normal ♀ 100	1.05

et al. (9). They are drawn from the first 17 cases in their series, with mean blood pressure greater than 120 mm. Hg, averaging the sets of basal observations for each individual. In every case, the afferent-to-efferent arteriolar ratio is

FIG. 1. EFFECT ON RENAL FUNCTION OF INTRAVENOUS ANGIOTONIN IN MAN

Afferent arteriolar constriction exceeds efferent arteriolar constriction as shown by the increase in their ratio (R_A/R_E).

greater than the normal one for men or women if the normal blood pressure is taken at 100 mm. Hg. A predominance of afferent arteriolar constriction seems outstanding in essential hypertension.

7. Renal function in late toxemia of pregnancy

Corcoran and Page (7) found that "decreased filtration fraction, whatever the level of renal blood flow, is frequently associated with a severe course and with eclampsia." Table V comprises this group with low filtration fraction where measurements of diodrast clearance (cc. per 1.73 sq. m. per minute) were made. The serum protein was subnormal and variable in these cases so that a correction for the consequent changes in blood viscosity has been made as described elsewhere (2).

Aside from the fall in renal blood flow along with decline in blood pressure, and the rise in filtration fraction and serum protein during recovery, we are impressed with the apparently high initial degree of afferent arteriolar constriction. The afferent-to-efferent arteriolar ratio declined in all cases except the one (No. 8) which developed persistent hypertension (Table V).

DISCUSSION

It does not appear that the action of pitressin, renin, or angiotonin can, at present, be considered confined either to afferent or efferent arterioles. Conclusions based on dialectic reasoning are not adequate in deciding whether a given change in renal function is due to change predominantly in the afferent or efferent arterioles. Where so many factors are involved (blood pressure, blood flow, filtration fraction, hematocrit, osmotic pressure, viscosity), such reasoning can break down and a mathematical method for revealing related manifestations becomes essential.

Perhaps the most outstanding application of formulas for renal arteriolar resistance is to the question of the relative importance of efferent arteriolar constriction in human hypertension. Since renal blood flow is only slightly reduced while filtration fraction is slightly increased in hypertension (9, 10), an examination of the formula for R_E shows that slight increase in efferent arteriolar resistance should be expected in the hypertensive. The blood pressure (P_M) does not even enter into the formula for R_E , but it appears so prominently in the one for R_A , that

TABLE V
Late toxemia of pregnancy

Case, mild or severe	Days ante- or post-partum	Glomerular filtration rate I	Blood flow HD	Filtration fraction $F=I/D$	Blood pressure P_M	Glomerular intracapillary pressure P_G	Serum protein S	Relative blood viscosity U	Afferent arteriolar hydraulic resistance R_a	Efferent arteriolar hydraulic resistance R_e	Total arteriolar hydraulic resistance R_t	Afferent resistance Efferent resistance R_a/R_e
		cc. per 1.73 square meters per minute	cc. per 1.73 square meters per minute	per cent	mm. Hg	mm. Hg	grams per 100 cc.	referred to water as unity	mm. Hg per liter per 1.73 square meters per minute referred to water			
1 S	20A	82	1050	12.5	155	44.2	5.8	3.28	26.3	3.93	30.2	6.71
	6P	105	907	20.0	135	47.9	5.8	3.54	20.9	5.11	26.0	4.08
	72P	92	675	23.0	120	72.6	7.8	4.24	9.57	9.64	19.2	0.99
4 S	18A	81	1065	12.1	145	35.6	4.3	3.82	22.0	2.88	24.9	7.64
	5P	93	705	22.5	125	38.6	4.3	2.88	32.8	6.8	39.6	4.81
	109P	130	850	23.8	108	82.2	8.3	4.13	1.65	9.34	11.0	0.176
6 S	3P	68	1075	10.5	130	45.3	6.1	3.53	17.1	3.51	20.6	4.85
	16P	62	436	23.0	100	62.0	7.0	3.75	11.0	14.0	25.0	0.785
	37P	79	595	22.0	98	61.0	7.0	3.80	75.2	9.76	85.0	0.771
(2 mos. preg.)	114P	104	985	17.0	87	56.4	7.0	3.87	2.77	4.84	7.6	0.573
8 M (HBP)	6P	92	1105	14.3	160	45.0	5.8	4.50	24.6	3.66	28.3	6.72
	16P	90	744	19.8	105	46.3	5.6	3.28	15.9	6.50	22.4	2.44
	64P	98	765	22.0	128	45.2	5.3	3.29	25.0	6.33	31.3	3.95
12 S	3A	103	1048	15.8	125	49.5	6.3	3.50	15.2	4.32	19.5	3.51
	12P	99	805	19.2	103	51.6	6.3	3.40	11.5	6.38	17.9	1.80
	112P	119	776	24.9	101	68.1	7.3	3.90	4.28	8.68	13.0	0.493

fractional changes in P_M produce proportionately larger changes in R_A , the afferent arteriolar resistance.⁴ Therefore, in hypertension, we should expect that R_A/R_E would be greater than normal (about 1.1) with afferent arteriolar constriction predominating. All the essential hypertensives here reported had high R_A/R_E , and so did the hypertensives who suffered from late toxemia of pregnancy, even when we restrict ourselves to the post-partum observations with normal rather than low filtration fractions. Further confirmation of this view of the importance of the afferent arterioles during blood pressure change in maintaining renal homeostasis is given by the data on R_A/R_E in the fall of blood pressure found in spinal anesthesia in man (1). There R_A/R_E dropped with fall in blood pressure, indicating that the afferent arterioles had dilated, with decreasing pressure, more than the efferent arterioles, in order to sustain glomerular intra-capillary pressure. Another example of the close connection between afferent arteriolar resistance and blood pressure is the series with atropine after pitressin. There, afferent constriction accompanied the rise in blood pressure which very likely was uncomplicated by any direct atropine effect on the kidney. Furthermore, in the anesthetized dog, it has been shown that kidney blood flow varies much less than blood flow in the hind limb when blood pressure is varied reflexly over a wide range (11, 12).

These divergent causes of abnormal blood pressure, with compensation by the afferent arterioles so as to maintain some measure of renal homeostasis, suggest that, if blood pressure rise were prevented during the infusion of renin and angiotonin, and if the purely physical effect of the elevation in pressure (as opposed to its pharmacological cause) in hypertension and toxemia could be abolished, we might discover a considerable measure of specificity in the site of action of the drugs and the disease agents.

The low filtration fraction in the severer cases of late toxemia of pregnancy is an interesting

finding. An examination of all the data (7) indicates that the cause is sometimes, in whole or in part, increased renal blood flow and sometimes, in whole or in part, the cause is a reduced rate of glomerular filtration. A low glomerular intra-capillary pressure can explain these various combinations of causes. In view of the high blood pressure, it is clear (both theoretically, from the formulas, and from R_A/R_E in Table V) that considerable afferent arteriolar constriction would be required.

Other possible explanations must also be explored. While they are not mutually exclusive, it is simpler to deal with them as though they were, remembering meanwhile that combinations are possible.

A simple explanation would be that inulin clearance ceases to be a measure of filtration rate in these toxemia cases (13, 14). If the glomerular membrane were affected (swelling?) so that inulin did not diffuse through as completely as water, its clearance would be less than the glomerular clearance of water. True filtration fraction would therefore be greater than our recorded one. The argument for the identity of inulin clearance and filtration rate rests, in part, on the equality of the clearance rates of certain hexitols with that of inulin (15). Wellen, Welsh, and Taylor, Jr. (14) have compared the simultaneous clearance of inulin with that of mannitol and sorbitol, of lower molecular weight, in 5 pre-eclamptic subjects and found them identical. Though diffusion of all the 3 sugars may be equally retarded by the diseased glomeruli, as compared to water, it does not seem especially likely in view of their differing molecular weights.

Let us now consider the possibility that harm to the glomerular membrane interferes with the establishment of osmotic equilibrium between glomerular filtrate and the blood leaving the glomerular capillaries (13). This is the same as saying that the diffusion of water through the glomerular capillaries is not completed, so far as osmotic equilibrium is concerned, before the blood leaves Bowman's capsule. Our formula assumes that such equilibrium exists. If it is not achieved, glomerular intra-capillary pressure is higher than we have supposed, and our afferent-to-efferent ratio is larger than it should be. If this possibility were in truth the case, we should

⁴ Blood flow rate appears in the denominator of both R_A and R_E which leaves their ratio unaffected. An increase in filtration fraction will increase $P_{O'}$, which will by itself tend to increase R_E and decrease R_A , but to a much lesser extent than the opposing influence of the large increase in P_M in hypertension.

expect a differential diffusion rate, not only between the slowed down water and the heavier inulin, but between inulin and the lighter sugars, which we have seen is not so.

While such an argument is not conclusive, it is at least presumptive that inulin clearance is a correct measure of filtration rate and that osmotic equilibrium is achieved in the glomerulus in toxemia of pregnancy.

Another workable hypothesis is that certain glomeruli, among many fairly normal ones, were completely impermeable to all the sugars, while permitting smaller molecules and water to pass. In this case, inulin clearance for the kidney as a whole would be less than actual glomerular filtration rate. If the transition stage from free passage of water plus all the sugars to water alone were rapid, very few glomeruli would be in the condition of differential filtration of sugars when clearances are measured clinically, so that the precision of measurement of clearance required to detect these few transitional glomeruli would be unobtainable.

This last suggestion emphasizes the peculiar significance of the formulas. They are not measuring the resistance of any particular arteriole. They are really measuring the resistance of the arterioles supplying an ideal functional nephron, which replaces the average of the millions of nephrons of the kidney. Where there is wide discrepancy between the function of the many nephrons in the kidney, as in a diseased state, the vagaries which may attend the usual meaning of inulin and diodrast clearances (13) are accentuated, and the broad integrating effect of the formulas requires evaluation.

Corcoran and Page (7), in considering the problem of the low filtration fraction in toxemia, write: "The decrease of filtration of water from plasma in these cases was due either to hemodynamic intrarenal changes resulting in decreased intraglomerular pressure . . . or to increased resistance of the filtering surface." They conclude, however, that their first alternative is untenable when renal blood flow is normal or subnormal and that only efferent arteriolar dilatation, and not afferent arteriolar constriction, is a possibility with increased renal flow. Consequently, preferring a single explanation as the more likely, and citing histological findings, and

not having the then unpublished evidence of the equal clearance of the hexitols, they argue in favor of a swelling of the glomerular membrane, whereby the passage of filtrate is impeded.

Dill *et al.* (16) decide against the notion of decreased glomerular membrane permeability, perhaps because of the albumin in the urine of the pre-eclamptic. It seems, however, quite possible for a membrane to have reduced permeability except for rare holes through which samples of all of the fluid, held back elsewhere, would pour. A clogged filter paper with a single pin-hole would be such an example.

As has been seen (Table V and also above), we feel it is quite possible for hemodynamic changes alone, exclusive of a pathological glomerular membrane, to explain the low filtration fraction in toxemia of pregnancy. The various ways in which the pathological glomerulus can interfere with the methods used have been discussed. Of them, the most appealing is the one which postulates some glomeruli, functional so far as water is concerned, but impermeable to all the hexitols. Can the idea of homeostasis as applied to the kidney help in distinguishing the most likely of the available alternatives?

In general, it appears that the kidney, like other organs in the body, acts in accordance with the principle of homeostasis. Its reaction to change in blood pressure has already been discussed. Especially does glomerular filtration rate tend to remain constant (17). Blood flow, also, seems to be more constant than in other organs (11, 12). When a system in stable equilibrium is disturbed, a new equilibrium is reached, near the first, in a direction such as to annul as far as possible, but not completely, that disturbance.⁵ This is really the biological analogue of the principle of mobile equilibrium of Le Chatelier (18). If the reduction in filtration fraction in toxemia of pregnancy minimizes, so far as renal homeostasis is concerned, the effects of increased blood pressure or reduced serum protein found in this disease, we should be more inclined to discount a specific renal effect in toxemia of pregnancy with low filtration fraction, whether on the permeability of the glomerular membrane or on the afferent arterioles. The

⁵ For example, acid added to a buffered solution shifts the hydrogen ion concentration slightly to the acid side.

observations would be explicable as a secondary renal response to physical changes in the blood—its increased arterial pressure and decreased protein content.

If we first consider increased blood pressure alone, we see from the formulas that, if the arterioles stay fixed, blood flow will be considerably increased. Furthermore, glomerular intra-capillary pressure will rise, which means that filtration fraction must have risen, according to the formula. Consequently, if homeostasis is the principle controlling the kidney during increase in blood pressure, we should expect the arterioles of the kidney to change their lumen so as to prevent a large increase in filtration fraction, but a *slight rise* is to be anticipated. This is the opposite of what we actually find in toxemia of pregnancy.

If serum protein falls while blood pressure and the arterioles are fixed, blood osmotic pressure falls so that a larger fraction of plasma than usual will be filtered before osmotic equilibrium is reached between glomerular filtrate and glomerular intra-capillary blood. That is to say, the filtration fraction (F) will rise. Equilibrium, then, if homeostasis is to occur in the face of hypoproteinemia, should also entail an increased filtration fraction.⁶

Thus, both the hypertension and the low blood protein in severe toxemia of pregnancy should lead to *high* rather than low filtration fraction, unless there is a specific renal effect of the toxemia. Since low rather than high filtration fraction has been found, we consider that a specific effect on the kidney is likely in toxemia of pregnancy, but we are not able to resolve the dilemma between a vascular cause—primarily afferent arteriolar constriction—and impairment of the glomerular membrane. It is not unlikely that both effects exist together.⁷

SUMMARY

Formulas for renal afferent and efferent arteriolar resistance have been applied to data in

⁶ The isolated kidney of the dog illustrates this, if it is comparable to the human kidney (19).

⁷ The increase in renal blood flow in the severe cases may well be, in part, a homeostatic mechanism to preserve adequate glomerular filtration rate in the face of a sluggish capillary filter.

the literature. It appears that dialectic reasoning concerning the predominance of afferent or efferent arteriolar constriction frequently leads to incorrect conclusions. Renal homeostasis and the importance of the afferent arterioles in protecting the kidney from blood pressure changes are discussed. The implications of the low filtration fraction observed in late toxemia of pregnancy are weighed.

CONCLUSIONS

1. Pitressin caused no consistent change in glomerular intra-capillary pressure, total effective renal arteriolar resistance, or in the afferent-to-efferent arteriolar resistance ratio in unanesthetized dogs.

2. Atropine added to pitressin increased total effective arteriolar renal resistance, with afferent arteriolar constriction predominating, in unanesthetized dogs.

3. Renin infused into unanesthetized dogs increased glomerular intra-capillary pressure and total arteriolar resistance with neither afferent nor efferent constriction predominating consistently.

4. Angiotonin acted rather similarly to renin, but conclusions concerning it are subject to some doubt.

5. In one test on a human subject, angiotonin caused constriction of both sets of arterioles with afferent constriction predominating.

6. Afferent arteriolar constriction outweighed efferent constriction more than is normal in all of the 17 cases of essential hypertension studied.

7. It is likely that the resistance of the afferent arterioles varies with blood pressure changes so as to preserve renal function.

8. A specific renal effect is the probable cause of the low filtration fraction seen in late severe toxemia of pregnancy. There is inadequate evidence to decide how much of this effect is primarily constriction of the efferent arterioles and how much, if any, is change in the permeability of the glomerular membrane to water and/or inulin and other sugars.

BIBLIOGRAPHY

1. Lamport, H., Formulae for afferent and efferent arteriolar resistance in the human kidney: an application

- to the effects of spinal anesthesia. *J. Clin. Invest.* 1941, 20, 535.
2. Larnport H., To be published.
 3. Corcoran, A. C., and Page, I. H., Effects of renin, pitressin, and pitressin and atropine on renal blood flow and clearance. *Am. J. Physiol.*, 1939, 126, 354.
 4. Corcoran, A. C., and Page, I. H., Effects of angiotonin on renal blood flow and glomerular filtration. *Am. J. Physiol.*, 1940, 130, 335.
 5. Corcoran, A. C., Kohlstaedt, K. G., and Page, I. H., Changes of arterial blood pressure and renal hemodynamics by injection of angiotonin in human beings. *Proc. Soc. Exper. Biol. and Med.*, 1941, 46, 244.
 6. Corcoran, A. C., and Page, I. H., Renal blood flow and sympathectomy in hypertension. *Arch. Surg.*, 1941, 42, 1072.
 7. Corcoran, A. C., and Page, I. H., Renal function in late toxemia of pregnancy. *Am. J. M. Sc.*, 1941, 201, 385.
 8. Adair, G. S., Adair, M. E., and Greaves, R. I. N., Osmotic pressure, after re-solution, of serum, dried from frozen state (F. D. serum). *J. Hyg.*, 1940, 40, 548.
 9. Goldring, W., Chasis, H., Ranges, H. A., and Smith, H. W., Effective renal blood flow in subjects with essential hypertension. *J. Clin. Invest.*, 1941, 20, 637.
 10. Smith, H. W., Studies in the physiology of the kidney. Porter Lecture Ser., No. 9, Lawrence, 1939.
 11. Hartmann, H., Ørskov, S. L., and Rein, H., Die Gefäßreaktionen der Niere im Verlaufe allgemeiner Kreislauf-Regulationsvorgänge. *Arch. f. d. ges. Physiol.*, 1936, 238, 239.
 12. Opitz, E., and Smyth, D. H., Nierendurchblutung bei Reizung des Carotis-Sinus. *Arch. f. d. ges. Physiol.* 1937, 238, 633.
 13. Smith, H. W., Note on interpretation of clearance methods in diseased kidney. *J. Clin. Invest.*, 1941, 20, 631.
 14. Wellen, I., Welsh, C. A., and Taylor, H. C., Jr., The filtration rate, effective renal blood flow, tubular excretory mass and phenol red clearance in specific toxemia of pregnancy. *J. Clin. Invest.*, 1942, 21, 63.
 15. Smith, W. W., Finkelstein, N., and Smith, H. W., Renal excretion of hexitols (sorbitol, mannitol, and dulcitol) and their derivatives (sorbiton, isomannide, and sorbide) and of endogenous creatine-like chromogen in dog and man. *J. Biol. Chem.*, 1940, 135, 231.
 16. Dill, L. V., Isenhour, M. A., Cadden, J. F., and Schaffer, N. K., Glomerular filtration and renal blood flow in the toxemias of pregnancy. *Am. J. Obs. and Gyn.*, 1942, 43, 32.
 17. Smith, H. W., and others, Glomerular dynamics in normal human kidney. *J. Clin. Invest.*, 1940, 19, 751.
 18. Glasstone, S., Text-Book of Physical Chemistry, New York, 1941.
 19. Eggleton, M. G., Pappenheimer, J. R., and Winton, F. R., Mechanisms of dilution diuresis in isolated kidney and anesthetized dog. *J. Physiol.*, 1940, 98, 336.

CAPILLARY BLOOD PRESSURE IN MAN. DIRECT MEASUREMENTS IN THE DIGITS DURING INDUCED VASOCONSTRICTION¹

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The present studies were undertaken because it was believed that direct measurements of capillary blood pressure in normal and hypertensive subjects might yield information concerning the nature of the peripheral resistance to blood flow in patients with hypertension. In man, only the capillaries of the digital nail-folds have been readily accessible for direct measurement of capillary blood pressure; but, digital circulation is very labile and readily affected by numerous influences (1, 2). Therefore, before proceeding to comparisons of digital capillary blood pressure in different individuals, it seemed necessary to know the extent to which such pressures are altered by those physiologic influences known to affect the digital circulation. This communication deals with the effects of one of these influences, namely, digital vasoconstriction.

METHODS

The methods will be considered under two headings: (1) General and (2) Particular. The *general* methods were employed uniformly in all of these studies of capillary blood pressure. The methods designated *particular* pertain only to the experiments of this publication.

General

The direct micro-injection method (Landis) (3), modified as previously described (4), was used to measure the capillary blood pressure in single capillaries in the nail folds of the fingers. The glass micropipette and the connecting manometer system were filled either with Ringer's solution or with 0.85 per cent sodium chloride solution, each containing heparin (3 mgm. per 100 cc.), and adjusted to a final pH between 7.3 and 7.4. Bubbles were carefully eliminated. The micropipette (orifice 6μ to 10μ in diameter) was inserted into the capillary in such a manner that the blood flow within the capillary was not visibly altered. When alteration of capillary blood flow occurred and persisted, observations were discontinued. Only measurements obtained while the blood

flow in the capillary remained visibly normal were recorded as capillary blood pressures. The pressure reading was made when equilibrium had been established between the pressure within the capillary and that within the micropipette. Equilibrium was indicated by the pulsatile oscillation of erythrocytes in the extreme tip of the micropipette but without progressive movement either into or out of the micropipette. A free communication between the lumina of micropipette and capillary was insured at all times, by repeatedly testing the rapidity with which the erythrocytes moved into or out of the tip of the micropipette when the pressure within the micropipette was altered from the equilibrium level by not more than 1 to 2 mm. Hg.

Approximately 1 hour before measurements of capillary blood pressure were begun, the most superficial, non-living, layer of the epidermis covering the nail fold was cut away with a keen razor blade. If viable tissue was cut and bleeding ensued, observations were not made on that nail fold.

All subjects lay supine, with the head elevated 10° to 15° . The nail fold was placed at a level approximating that of the right auricle, i.e., 3 to 6 cm. posterior to the angle of Louis. In no subject was there an increase in systemic venous pressure, as judged by distension of the superficial veins. Except when purposely altered, the observations were made in a warm room (23° C. to 28° C.). The temperature of the body was measured usually by a mouth-thermometer, occasionally by an indwelling rectal thermocouple. Digital skin temperature was determined at frequent intervals by means of copper-constantan thermocouples, constantly in contact with the pads of the digits. The digital skin temperature was not uniform in all subjects, but throughout a single experiment it remained fairly constant. In most observations, it was between 30° C. and 33° C., a state hereafter referred to as *moderate digital vasodilatation*.

Brachial arterial pressure was determined by the usual auscultatory method (mercury manometer) at the beginning and end of each experiment, and often more frequently. These measurements were made in the extremity in which capillary blood pressure was determined. After each measurement of arterial pressure, sufficient time was allowed for recovery from reactive hyperemia before capillary blood pressure determinations were resumed. For the sake of convenience, "mean" arterial pressure was assumed to be one-half of the sum of the systolic and diastolic pressures.

Reflex vasodilatation was produced by warming the body of the subject until sweating was profuse and the

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temperature of the exposed digits reached 33° C. to 35° C. In these experiments, the temperature of the room was maintained at about 20° C.

When histamine was employed to produce local vasodilatation, histamine acid phosphate (diluted 1:100 with 0.85 per cent salt solution) was pricked into the dorsum of the finger in three sites, each 0.5 cm. proximal to the nail-fold. Capillary blood pressure was measured when the resulting local erythema was pronounced, usually 5 to 12 minutes (and never later than 24 minutes) after pricking in the histamine.

Reactive hyperemia of the digit was produced by releasing the digital circulation after it had been completely arrested for either 5 or 10 minutes. The circulatory arrest was obtained by inflating, to pressures well above the systolic arterial pressure, a pneumatic cuff, encircling either the forearm or the base of the digit. The capillary blood pressure was determined as quickly as possible after the release of the circulation: within 30 to 40 seconds after a 5 minute period of ischemia; within 60 seconds after a 10 minute period of ischemia.

Interruption of the sympathetic innervation of the digits was accomplished either (a) temporarily, by the injection of 2 per cent procaine into the region of the stellate and upper thoracic sympathetic ganglia, or (b) permanently, by preganglionic sympathectomy of the upper extremity by the method of Smithwick (5, 6). The adequacy of the sympathetic denervations was confirmed by the absence of vasoconstrictions of the neurogenic type in response to stimuli which induced vasoconstrictions in a normally innervated digit of the same subject. At least 7 to 10 days elapsed between operative sympathectomy and determination of capillary blood pressure in the sympathectomized digits.

Particular

The determinations of capillary blood pressure before, during, and after vasoconstriction were made while the micropipette was constantly in the same location in a given capillary. One observer continuously adjusted the pressure in the micropipette, keeping it always in equilibrium with the pressure in the capillary. Whenever equilibrium points were established (5 to 15 second intervals), this observer signaled an assistant, who read the pressure in the manometer system connected with the micropipette. During such a series of readings, a third observer administered vasoconstrictor stimuli. Often, the nature of the stimulus and the time of its application were unknown to the observer adjusting the pressure in the micropipette.

Vasoconstrictions in the digits, *i.e.*, reductions in digital volume (and presumably in blood flow), were induced by the following stimuli: touching the skin of a remote area (*e.g.*, face, shoulder, or leg) with ice, or pricking it with a sharp object, having the subject take a deep breath, or solve mentally a problem in arithmetic. Vasoconstrictions caused by such stimuli are known to be mediated over sympathetic nervous pathways (7), and are here termed *neurogenic*. Digital vasoconstrictions

were also induced by the intravenous injection of epinephrin hydrochloride in doses of 1 to 2.5 gamma (0.1 to 0.25 cc. of a 1:100,000 solution).

Changes in digital volume during vasoconstrictions were recorded optically by the plethysmographic method of Bolton, Carmichael, and Stürup (8). Plethysmographs were applied usually to 2 digits: (1) a finger, usually the thumb, of the hand in which capillary blood pressure was being determined; (2) that finger of the opposite hand, corresponding to the one serving for measurements of capillary blood pressure. Occasionally, the great toe of one foot was substituted for one of the fingers.

Most of the subjects were young adults. One group comprised normal and hypertensive subjects whose capillaries were of normal size. With normal capillaries, it was technically difficult to record continuously the capillary blood pressure for sufficiently long periods of time. Many additional observations were, therefore, made in a group of patients having Raynaud's disease or scleroderma. Their abnormally large capillaries presented fewer technical difficulties. Only in the latter subjects was capillary blood pressure correlated with simultaneous graphic recording of digital vasoconstrictions.

RESULTS

A. Subjects with capillaries of normal size

In 3 normal and 4 hypertensive subjects, the capillary blood pressure was determined before and during stimuli known to induce neurogenic vasoconstrictions (Table I). After the administration of each stimulus, there occurred a temporary fall in the capillary blood pressure, followed by a return to the initial level. At times, the capillary blood pressure measured 5 to 10 seconds after the application of the stimulus was not altered from the initial level; but the second reading, 10 to 15 seconds after the stimulus, was invariably lowered. The duration (10 to 55 seconds) and magnitude (2 to 10 mm. Hg, 5 to 33 per cent) of these falls in capillary blood pressure were approximately equal in both normal and hypertensive subjects (Table I).

In 2 subjects, one normal, the other hypertensive, the observations were made during reflex vasodilatation. In each subject, decreases in capillary blood pressure followed the administration of the stimulus; in fact, the greatest falls (8 mm. Hg and 10 mm. Hg) occurred in these subjects (Table I).

TABLE I

Change in capillary blood pressure following "neurogenic" vasoconstrictor stimuli. Normal sized capillaries. Intact innervation

Subject (Sex, age)	Arterial pressure	Skin tem- per- ature	Location in capillary where pressure was measured	Capillary blood pressure		Duration of change in cap- illary blood pres- sure	Stimulus	Remarks	
				Initial	Maximum change due to stimulus				
NORMAL SUBJECTS									
	mm. Hg	° C.		mm. Hg	mm. Hg	Per cent of initial	seconds		
C. J. (F, 38)	148/94	31.6	Venous limb	20.5	-3.5	-17.1	15	Deep breath	
	148/94	31.6	Venous limb	22.0	-3.0	-13.6	40	Deep breath	
	148/94	31.6	Venous limb	20.5	-2.5	-12.2	15	Pin prick	
R. H. (M, 26)	124/68	33.5	Arteriolar limb	31.5	-8.5	-27.0	45	Deep breath	Reflex vasodilatation
M. E. (F, 32)	116/76	32.3	Summit	29.0	-6.0	-20.7	55	Instructions before deep breath	
Average—Normals					-4.7	-18.1	34		
HYPERTENSIVE SUBJECTS									
J. A. (M, 41)	148/106	31.4	Venous limb	20.0	-5.5	-27.5	45	Deep breath	
C. B. (M, 35)	222/148	35.5	Arteriolar limb	57.0	-5.0	- 8.8	50	Pin prick	
E. B. (F, 35)	232/172	33.0	Venous limb	20.0	-6.0	-30.0	30	Deep breath	24 minutes after intradermal histamine (1 : 100) Reflex vasodilatation Reflex vasodilatation
	232/172	33.2	Summit	40.0	-2.0	- 5.0	?	Deep breath	
	218/154	33.2	Arteriolar limb	28.0	-8.0	-28.6	15	Pin prick	
	220/160	33.4	Arteriolar limb	30.0	-10.0	-33.3	10+	Deep breath	
H. B. (M, 35)	178/136	33.6	Venous limb	22.0	- 5.0	-22.7	15	Deep breath	16 minutes after intradermal histamine (1 : 100)
	178/136	33.6	Venous limb	21.0	- 5.0	-23.8	15	Deep breath	20 minutes after intradermal histamine (1 : 100)
Average—Hypertensives					- 5.8	-22.4	22		
Average of all					- 5.4	-20.8	29		

In 2 hypertensive subjects with moderate digital vasodilatation, marked vasodilatation was induced locally in the nail fold by pricking in histamine. Vasoconstrictor stimuli applied at the height of the resulting erythema were still followed by decreases (2 mm. Hg to 5 mm. Hg) in the capillary blood pressure (Table I).

The above changes in capillary blood pressure were never accompanied by visible alterations in the diameter of the capillaries or in the flow of blood through them.

B. Subjects with abnormally large capillaries

Each of the 7 patients in this group had either Raynaud's disease, or scleroderma, or both, involving the fingers. Only in subject M. B. were the capillaries of approximately normal size; in all other subjects, they were unquestionably abnormally large.

1. Intact sympathetic innervation

Neurogenic vasoconstriction. With the digital circulation in moderate vasodilatation, neurogenic

vasoconstrictor stimuli were administered 89 times to 7 subjects (Table II). Following 52 stimuli, the capillary blood pressure decreased (Figure 1A, Figure 2A) by 1 mm. Hg to 8 mm. Hg (4.4 to 27.8 per cent). These falls in pressure persisted for 5 to 65 seconds. After 29 stimuli the capillary blood pressure remained essentially unchanged (Figure 3); and on the remaining 8 occasions, it rose slightly (1 mm. Hg to 2 mm. Hg). The stimuli which were followed by no change or a slight rise in capillary blood pressure, usually induced slight digital vasoconstrictions of short duration, or none at all. On one occasion, however, an increase in pressure of 5 mm. Hg accompanied a substantial vasoconstriction. Two subjects (M. S. and M. B.) accounted

for 7 of the 8 instances in which the capillary blood pressure rose during digital vasoconstriction.

Usually, the more marked decreases in capillary blood pressure accompanied the more marked and more prolonged vasoconstrictions (Table II). There were exceptions. For example, in patient C. M., a deep breath was, on one occasion, followed by a fall of 8 mm. Hg in the capillary pressure, although the simultaneously recorded digital vasoconstriction was slight. In patient F. G., ice applied to the face induced, on one occasion, a marked digital vasoconstriction, during which the capillary blood pressure remained unaltered.

Although vasoconstrictions occasionally were not accompanied by significant changes in capillary blood pressure (Figure 3), decreases in capil-

TABLE II
Change in capillary blood pressure during vasoconstriction induced by "neurogenic" vasoconstrictor stimuli.
Dilated capillaries. Intact innervation

Subject (Sex, age)	Diagnosis	Arterial pres- sure	Skin temper- ature	Location in capillary where pressure was measured	Capillary blood pressure			Dura- tion of change in capil- lary blood pressure	Magni- tude of vasocon- striction by plethysmo- graphy	Stimulus	Remarks
					Initial	Maximum change due to stimulus					
		mm. Hg	° C.		mm. Hg	mm. Hg	per cent of initial	seconds			
T. A. (M, 26)	Scleroderma	90/56	31.1	Venous limb	18.5	0	0			Ice to leg Deep breath Deep breath Ice to leg Ice to leg Ice to leg Deep breath Deep breath Mental problem Deep breath Deep breath Ice to leg Ice to neck Ice Pin prick Ice Pin prick Mental problem	
				Venous limb	18.5	-3	-16.2	57			
				Venous limb	18.5	-2	-10.8	65			
				Arteriolar limb	22	-3.5	-15.9	35			
				Arteriolar limb	22	-4	-18.2	60			
				Arteriolar limb	21	-2	-9.5	35			
				Arteriolar limb	22	-5	-22.7	15+			
				Arteriolar limb	22	-5	-22.7	51			
				Arteriolar limb	22	0	0				
				Arteriolar limb	23	-3.5	-15.2	35			
		94/58	30.8	Venous limb	13	-2	-15.4	30			After intradermal histamine (1 : 100)
				Summit	14	-1	-7.1	7			
				Summit	14	-1.5	-10.7	15			
				Summit	14	0	0				
				Summit	14	0	0				
				Summit	15.5	-3	-19.3	50	3+		
		102/72	28.6	Summit	15	-2	-13.3	15	2+		
				Summit	15	-2.5	-16.6	45	3+		
				Venous limb	16	-2	-12.5	40	2+		
				Venous limb	18	-1	-5.6	?	1+		
B. B. (F, 44)	Scleroderma with Raynaud's Disease	110/76	25.3	Venous limb	17	-4	-23.5	35		Ice to leg Pin prick Deep breath Deep breath Ice to leg Deep breath	
				Venous limb	16.5	0	0				
				Venous limb	16.5	-3	-18.2	20?			
				Summit	19	-4	-21.0	15			
				Summit	18	-3	-16.7	15			
				Summit	20	-5	-25.0	15			
M. B. (F, 47)	Scleroderma with Raynaud's Disease	124/86	25.0	Venous limb	17	+5	+29.4	22+	2+	Pin prick Ice to face	Capillaries of "top normal" size
				Venous limb	19	+2, -2.5	+10.5 -13.1	10 15	2+		
M. S. (F, 17)	Raynaud's Disease	86/50	30.0	Venous limb	18	-0.5	-2.8	15	?	Ice to shoulder Deep breath Pin prick Ice to neck Ice to shoulder Deep breath Pin prick Deep breath Ice to neck Deep breath Pin prick Mental problem Deep breath Deep breath	
				Venous limb	18	0	0		±		
				Venous limb	18	+1	+5.6	6	1+		
				Venous limb	18	+1	+5.6	10	1+		
				Venous limb	18	+0.5	+2.8		±		
				Venous limb	19	-0.5	-2.6		1+		
				Venous limb	18.5	+0.5	+2.7		±		
				Venous limb	18	+1	+5.6	15	1+		
				Venous limb	19	+1	+5.3	10	1+		
				Venous limb	18	+2	+11.1	15	±		
				Venous limb	18.5	0	0		0		
				Venous limb	18.5	+0.5	+2.7	10	1+		
				Venous limb	17	-3	-17.6	25	±		
				Venous limb	16	0	0		±		

CAPILLARY BLOOD PRESSURE IN INDUCED VASOCONSTRICTION

TABLE II—Continued

TABLE II—Continued												
Subject (Sex, age)	Diagnosis	Arterial pres- sure	Skin temper- ature	Location in capillary where pressure was measured	Capillary blood pressure			Dura- tion of change in cap- illary blood pressure	Magni- tude of vasocon- striction by ple- thysmo- graphy	Stimulus	Remarks	
					Initial	Maximum change due to stimulus						
		mm. Hg	° C.		mm. Hg	mm. Hg	per cent of initial	seconds				
F. H. (M, 38)	Scleroderma	118/62	30.7	Venous limb	22.5	-4.5	-20.0	25	2+	Ice to foot Pin prick Ice Pin prick Ice to face Ice to foot		
				Venous limb	23	-3	-13.0	25	1+			
				Venous limb	22	-4	-18.2	40	2+			
				Summit	23	-1	-4.4	5	0			
				Venous limb	24	-5	-20.8	45	3+			
				Venous limb	23	-2.5	-10.9	10	2+			
C. M. (F, 29)	Raynaud's Disease	128/86	27.0	Venous limb	41	0	0		0	Ice to face Pin prick Ice to face Ice to foot Ice to face Deep breath Ice Deep breath Deep breath Deep breath Pin prick Deep breath Deep breath Deep breath Ice Deep breath Deep breath Deep breath		
		Summit	27	0	0		0					
		128/86	32.1	Summit	29	0	0		0			
				Summit	28	0	0		0			
				Summit	29.5	+1.5	+ 5.1	730	1+			
				Summit	28	-3	-10.7	15	±			
				Summit	32	0	0		2+			
				Summit	30	-3.0	-10.0	25	0			
				Summit	30	-3.5	-11.7	25	1+			
				Summit	30	-3.5	-22.8	30	±			
				Summit	35	-8	-22.8		±			
				Summit	32	0	0		0			
				Summit	31	-4	-12.9	30	2+			
				Summit	26.5	-3	-11.3	15	±			
				Venous limb	30	0	0		0			
				Venous limb	26	0	0		1+			
				Venous limb	26	-2	-7.7	?	±			
				Venous limb	23	+2	+ 8.7	15	0			
				Venous limb	22	0	0		0			
				F. G. (M, 32)	Raynaud's Disease	126/82	32.4	Arteriolar limb	18			-5
118/78	33.4	Venous limb	19			-3	-15.8	35				
130/82	30.1	Venous limb	19			-2.5	-13.1	20				
		Venous limb	20			-3	-15.0	210	1+			
		Venous limb	19			0	0		2+			
		Venous limb	19			0	0		2+			
		Venous limb	18			-2	-11.1	0	1+			
		Venous limb	19			0	0		1+			
		Venous limb	17			-2	-11.7	15	2+			
		Venous limb	17			-3	-15.8	40	2+			
		Venous limb	19			-3	-15.8	20	2+			
		Venous limb	19			0	0		1+			
		Venous limb	18			0	0		2+			
		Venous limb	21			-1.5	-7.1	15	2+			
		Venous limb	20.5			0	0		3+			
		Venous limb	19			0	0		2+			
		Venous limb	19			0	0		3+			
		Venous limb	20			-3	-15.0	10	2+			
		Venous limb	19			-1	-5.3	35	2+			
		Venous limb	19			-1	-17.7	40	3+			
		Venous limb	17			-3	-17.7		0			
		Venous limb	16.5			0	-11.8	25	2+			
		Venous limb	17			-2	-8.8	25	3+			
		Venous limb	17			-1.5	-8.8	?	2+			
Venous limb	17	-1.5	-8.8			?	2+					
Venous limb	16	-2	-12.5			15	2+					

lary blood pressure were unusual in the absence of demonstrable vasoconstrictions.

During similar states of the digital circulation, as judged by the digital skin temperature, a given vasoconstrictor stimulus did not always induce the same degree of change either in the digital volume or in the digital capillary blood pressure. This maintained for different individuals and even in a series of observations on a single capillary (Table II).

Vasoconstriction induced by epinephrin injected intravenously. Epinephrin hydrochloride (1 to 2.5 gamma) was injected intravenously into 5 subjects. Strong digital vasoconstrictions followed 10 of the 11 injections; once the response was equivocal. A fall in capillary blood pressure

accompanied 7 of the 10 vasoconstrictions (Table III, Figure 1B). Between injection and initiation of vasoconstriction, 10 to 45 seconds elapsed (Figure 1B). This probably represented the time required for the epinephrin to reach the digit. The magnitude and duration of the decreases in digital volume and the associated falls in capillary blood pressure (1.5 to 22 mm. Hg, 12.5 to 58.5 per cent) (Table III) appeared to be more marked than those observed after neurogenic vasoconstrictor stimuli.

Small rises in the capillary blood pressure (1 mm. Hg to 2.0 mm. Hg) occurred with 3 of the 10 vasoconstrictions. Twice this occurred in subject M. S., in whom slight rises in capillary blood pressure also accompanied neurogenic vasoconstrictions.

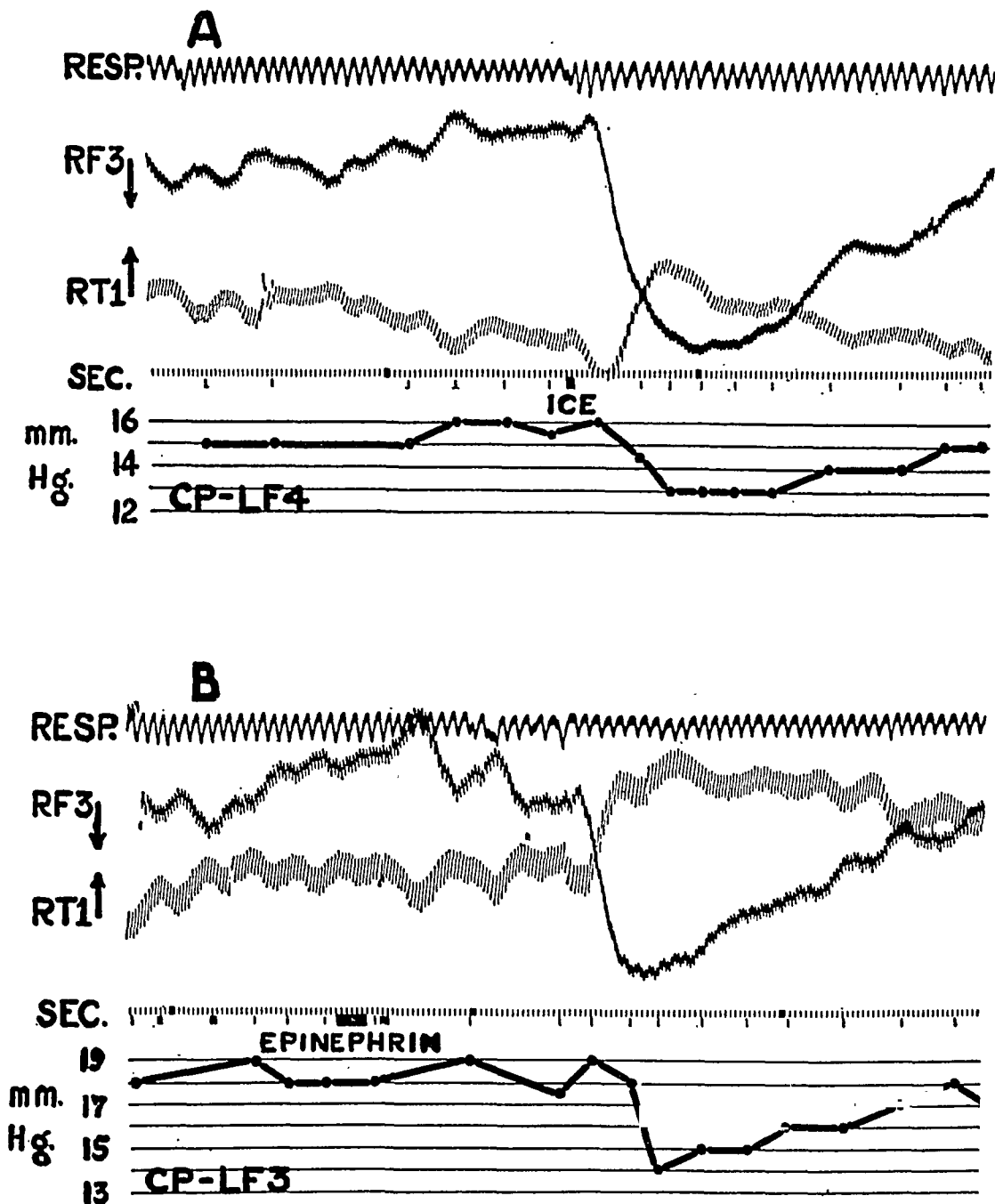


FIG. 1. SUBJECT T. A. MODERATE SCLERODERMA OF HANDS. INNERVATION OF ALL DIGITS INTACT IN BOTH A AND B

A. Simultaneous changes in capillary blood pressure and digital volume during vasoconstriction induced by ice to the skin. Capillary blood pressure measured in the summit of a capillary loop. Skin temperature LF 4, 27.5° C. Room temperature, 23.7° C. Arterial pressure, left arm 102/72 mm. Hg.

B. Simultaneous changes in capillary blood pressure and digital volume during vasoconstriction induced by the intravenous injection of 1 gamma of epinephrin hydrochloride. Capillary blood pressure measured in the venous limb of a capillary loop. Skin temperature LF 3, 30° C. Room temperature, 24.6° C. Arterial pressure, left arm 104/72 mm. Hg. (3 signal marks indicate beginning of "needling"; 4 marks, entry of needle into vein; solid mark, injection; 5 marks, withdrawal of the needle.)

All figures are similarly plotted and labelled. From above downward they record respectively: RESP.—respiration, the down stroke indicating inspiration. RF3↓, RT1↑ (or other letters)—digital pulse wave and digital volume, the arrow indicating the direction of a decrease in volume. SEC.—time interval in seconds, the solid mark indicating the minute

strictions. During the one equivocal response, the capillary blood pressure fell 3 mm. Hg.

2. Sympathetic innervation interrupted

Neurogenic vasoconstriction. Except on one occasion, when a small digital vasoconstriction followed the stimulus of mental arithmetic,² neurogenic vasoconstrictor stimuli (13 in all) failed to induce any change in the capillary blood pressure or in the volume of the sympathectomized digits of three subjects (Table IV, Figure 2B). During the one small vasoconstriction, capillary blood pressure increased 1 mm. Hg. In each subject, the same stimuli produced definite vasoconstrictions in the contralateral, normally innervated digits (Figure 2B).

Vasoconstriction induced by epinephrin injected intravenously. Epinephrin hydrochloride (1 to 2 gamma) was administered intravenously 9 times to 3 subjects, with temporarily or permanently interrupted sympathetic innervation of the digits (Table V). On each occasion, after the usual latent period, similar decreases in digital volume were recorded in both the sympathectomized and in the contralateral, normally innervated digits. In the sympathectomized digits, the vasoconstrictions were accompanied by falls in capillary blood pressure which in duration (15 to 60 seconds) and magnitude (1 mm. Hg to 11 mm. Hg, 6.3 to 35.5 per cent) approximated those observed in the normally innervated digit (Table V, Figure 4A, Figure 4B).

C. Relation between capillary blood pressure and changes in volume of digit

Although usually paralleling the decreases in digital volume, the falls in capillary blood pressure

²Recent evidence (9) indicates that a humoral (epinephrin-like) component may be involved in the production of the digital vasoconstriction induced by mental arithmetic. The effect of epinephrin in sympathectomized digits is discussed subsequently.

appeared at times to lag behind the changes in volume. Thus, during the initial phase of vasoconstriction, when the digital volume was decreasing most rapidly, the capillary blood pressure occasionally remained unchanged, or even increased slightly (Figure 1B). As the digital volume reached its lowest level, the capillary blood pressure also fell but occasionally did not attain its lowest value until after the digital volume had begun to increase toward its original level. The capillary blood pressure returned to its initial value more slowly than the digital volume. Such lags in capillary blood pressure behind the changes in digital volume were observed during vasoconstrictions induced by both epinephrin and neurogenic vasoconstrictor stimuli, and in sympathectomized as well as in normally innervated digits.

In all of the experiments, the changes in capillary blood pressure tended to be qualitatively the same in all parts of the capillary: arteriolar limb, summit, and venous limb.

DISCUSSION

It has previously been shown that digital capillary blood pressure is readily altered by changes in both the local (3, 4) and systemic (10) venous pressures, by the injection of histamine locally (3), and by the local application of heat and cold (3). Employing direct measurement, this study adds information concerning the effect upon capillary blood pressure of vasoconstrictions caused by reflex nervous mechanism and by a circulating pressor substance. This information was desired before proceeding to a comparison of capillary blood pressure in normal and hypertensive subjects (11).

Although capillary blood pressure in the nail-folds did fall during neurogenically induced digital vasoconstrictions, these reductions in pressure were relatively small: never exceeding 33.3 per cent of the initial value. Rarely, did the resultant

interval. The signal marks immediately below the time tracing indicate the exact time of a capillary blood pressure determination (single thin mark) and the time and duration of the administration of the stimulus noted immediately below (solid mark). All of the above is the actual optical record. Below this record and upon the same time axis is plotted the capillary blood pressure (CP—LF4, or similar legend) in mm. Hg. Each plotted point of capillary blood pressure represents the pressure at the time of the signal mark immediately above.

Digits are identified by the recognized scheme: the first letter indicating the side (right or left); the second letter, the digit (finger or toe) and the number the specific digit (first, second, etc.).

capillary blood pressure fall beyond the limits obtained under resting conditions. When compared with the reductions induced in digital blood flow during similar vasoconstrictions, the percentile decreases in capillary blood pressure are considerably smaller. For example, Wilkins, Doupe, and Newman (1) found that during neurogenic

vasoconstrictions "the flow to the fingers may be temporarily decreased as much as 20 times," and Burton (2) states that "a deep breath causes momentary almost complete cessation of flow in the fingers." Conversely, during reflex vasodilatation the blood flow to the fingers may increase as much as 100 times (1), but during similar condi

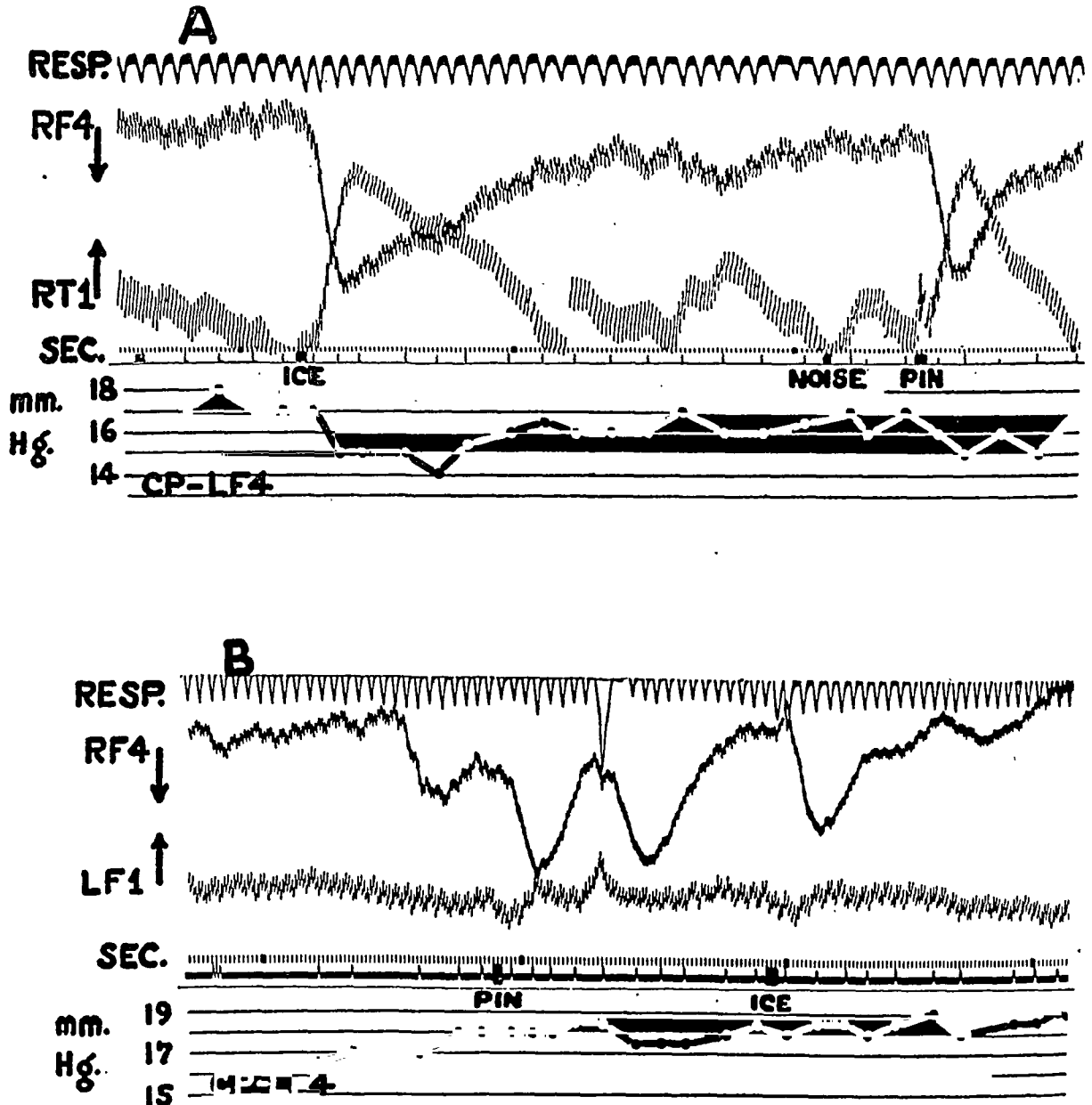


FIG. 2. SUBJECT F. G. RAYNAUD'S DISEASE OF HANDS AND FEET

A. Simultaneous changes in capillary blood pressure and digital volume during vasoconstrictions induced by ice and pin prick. Digital innervation intact. Capillary blood pressure measured at junction of venous limb and summit of a capillary loop. Skin temperature LF4, 30° C. Room temperature, 25.5° C. Arterial pressure left arm 122/88 mm. Hg.

B. Simultaneous observations of capillary blood pressure in a sympathectomized digit and of digital volume in a sympathectomized and in a normally innervated digit, during application of vasoconstrictor stimuli. Preganglionic sympathectomy of left upper extremity. Intact innervation right upper extremity. Capillary blood pressure measured at junction of venous limb and summit of a capillary loop. Skin temperature LF4, 31.5° C. Room temperature, 26.3° C. Arterial pressure, left arm 110/80 mm. Hg. Note spontaneous deep breath between stimuli of pin prick and ice.

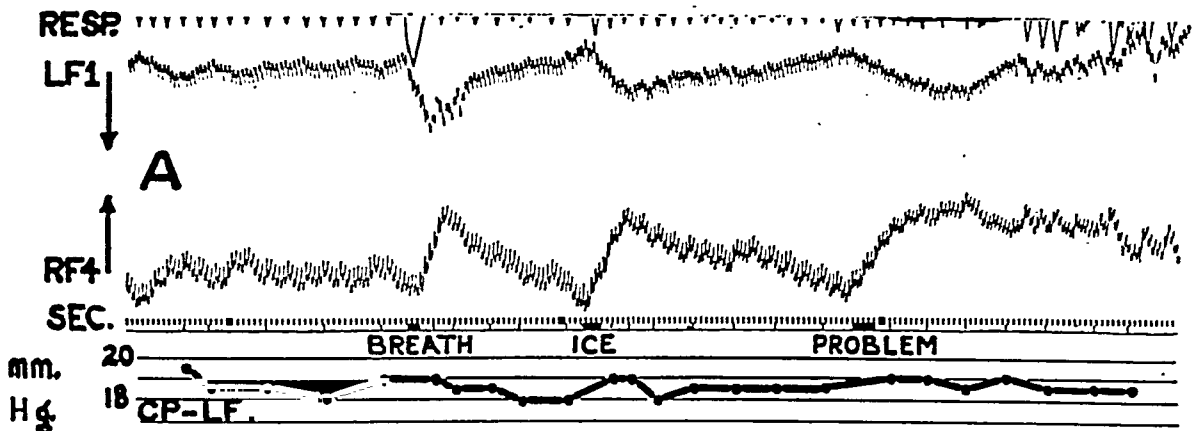


FIG. 3. SUBJECT M. S. RAYNAUD'S DISEASE WITH SCLERODERMA OF HANDS AND FEET

Simultaneous observations of capillary blood pressure and of digital volume during vasoconstrictions induced by deep breath, ice to the skin, and mental arithmetic. Digital innervation intact. Capillary blood pressure measured in the venous limb of a capillary. Skin temperature LF4, 29.2° C. Room temperature 24.5° C. Arterial pressure, left arm 86/50 mm. Hg.

tions no comparable increase in capillary blood pressure was found in this or in other studies (11).

There appears to be a homeostatic mechanism which keeps the digital capillary blood pressure within relatively narrow limits, even though blood flow in the digits is undergoing wide fluctuations

during digital vasoconstriction. The arteriole-venule anastomoses in the digits may contribute one component to this mechanism. By shunting blood through these structures, and by-passing the capillaries, wide variations in digital blood flow could occur without great changes in capillary blood pressure.

TABLE III

Change in capillary blood pressure during vasoconstriction induced by epinephrin intravenously. Abnormally large capillaries. Intact innervation

Subject (Sex, age)	Diagnosis	Arterial pres- sure	Skin temper- ature	Location in capillary where pressure was measured	Capillary blood pressure			Duration of change in capillary blood pressure	Magni- tude of vasoco- striction	Stimulus
					Initial	Maximum change due to stimulus				
		mm. Hg	° C.		mm. Hg	mm. Hg	per cent of initial	seconds		
T. A. (M, 26)	Scleroderma	102/72	28.5	Summit	19	-10	-52.7	98+	4+	Epinephrin 2γ
		102/72	28.5	Summit	18	- 3	-16.7	21	3+	Epinephrin 1γ
		102/72	28.5	Venous limb	16	- 5+	-31.2	15+	4+	Epinephrin 2.5γ
		102/72	28.5	Venous limb	18	- 4	-22.2	40	3+	Epinephrin 1γ
M. B. (F, 47)	Scleroderma Raynaud's Disease	124/86	25.0	Venous limb	37.5	-22?	-58.8	60+	2+	Epinephrin 1γ
F. H. (M, 38)	Scleroderma	118/62	30.7	Summit	38	- 8	-21.0	10+	3+	Epinephrin 2γ
M. S. (F, 17)	Raynaud's Disease	86/50	30.0	Venous limb	16	+ 1.5	+ 9.4	?	1+	Epinephrin 1γ
		86/50	30.0	Venous limb	15	+ 2	+13.3	?	2+	Epinephrin 1γ
C. M. (F, 29)	Raynaud's Disease	128/86	27.0	Summit	16	- 3	-18.7	?	?	Epinephrin 1γ
		128/86	32.1	Venous limb	20	+ 2	+10.0	25+	2+	Epinephrin 1γ
		128/86	32.1	Summit	24	- 3	-12.5		1+	Epinephrin 1γ
Average						- 4.8	-18.3	38.5		

TABLE IV

Capillary blood pressure during "neurogenic" vasoconstrictor stimuli. Abnormally large capillaries. Sympathetic innervation interrupted

Subject (Sex, age)	Diagnosis	Arterial pres- sure	Skin tem- pera- ture	Location in capillary where pressure was measured	Capillary blood pressure			Dura- tion of change in cap- illary blood pressure	Magni- tude of vasocon- striction	Stimulus	Remarks
					Initial	Maximum change due to stimulus					
		mm. Hg	° C.		mm. Hg	mm. Hg	per cent of initial	seconds			
F. H. (M, 38)	Scleroderma	118/62	30.7	Summit	36.5	-2.5	-6.8	?	0	Ice to leg	Paravertebral block. Capillary pressure remained at lower figure
M. S. (F, 17)	Raynaud's Disease	86/50	33.9	Summit	17	0	0	54	0	Ice to shoulder	Paravertebral block
		86/50	33.9	Summit	18	0	0		0	Pin prick	
		86/50	33.9	Summit	18	0	0		Moved	Deep breath	
		86/50	33.9	Summit	17.5	0	0		0	Ice to shoulder	
		86/50	33.9	Summit	17.5	0	0		0	Pin prick	
		86/50	33.9	Summit	17	0	0		0	Deep breath	
		86/50	33.9	Summit	17	0	0		Moved	Ice	
		86/50	33.9	Summit	17	+1.0	+5.9		1+	Mental problem	
F. G. (M, 32)	Raynaud's Disease	110/80	31.9	Summit	18	0	0		0	Pin prick	Preganglionic sympathectomy
		110/80	31.9	Summit	18.5	0	0		0	Ice to face	
		110/80	31.9	Summit	18.5	0	0		0	Ice	
		110/80	31.9	Summit	21.5	0	0		0	Pin prick	
		110/80	31.9	Summit	21.5	0	0		0	Ice	

TABLE V

Change in capillary blood pressure during vasoconstriction induced by epinephrin intravenously. Abnormally large capillaries. Sympathetic innervation interrupted

Subject (Sex, age)	Diagnosis	Arterial pres- sure	Skin tem- pera- ture	Location in capillary where pressure was measured	Capillary blood pressure			Dura- tion of change in capil- lary blood pres- sure	Magni- tude of vaso- con- stric- tion	Stimulus	Remarks
					Initial	Maximum change due to stimulus					
		mm. Hg	° C.		mm. Hg	mm. Hg	per cent of initial	seconds			
M. S. (F, 17)	Raynaud's Disease	86/50	33.9	Summit	16	- 1	- 6.3	15	1+	Epinephrin 1γ	Paravertebral block
		86/50	33.9	Summit	18	- 3.5	- 19.4	40	2+	Epinephrin 1γ	Block wearing off
C. M. (F, 29)	Raynaud's Disease	128/86	33.5	Arteriolar limb	32.5	?	?		2+	Epinephrin 1γ	Paravertebral block
		128/86	33.5	Arteriolar limb	25.5	- 2	- 7.8	15	2+	Epinephrin 2γ	Preganglionic sympathectomy
		112/76	33.0	Summit	24	+13.5 then -2	+56.3 - 8.3	30	2+	Epinephrin 1γ	
		112/76	35	Venous limb	29	- 4	- 13.8	35	1+	Epinephrin 1γ	
		112/76	35	Venous limb	31	- 11	- 35.5	25+	2+	Epinephrin 1γ	
F. G. (M, 32)	Raynaud's Disease	110/80	33.5	Venous limb	19.5	- 3.5	- 18.0	60	4+	Epinephrin 1γ	Preganglionic sympathectomy
		110/80	33.5	Summit	20	- 4.0	- 20.0	30+	4+	Epinephrin 1γ	
Average						- 2.6	- 16.1	31+			

Although of smaller magnitude, the falls in capillary blood pressure appeared to be directly related to the reductions in digital volume, and presumably in blood flow. Stimuli failing to induce reductions in digital volume caused no change in the capillary blood pressure; this was observed

particularly after interruption of the sympathetic nervous pathways. Unless these pathways were intact, the capillary blood pressure was not altered by such neurogenic stimuli as the application of ice to a remote area of skin, pricking the skin with a pin, or the taking of a deep breath. But even

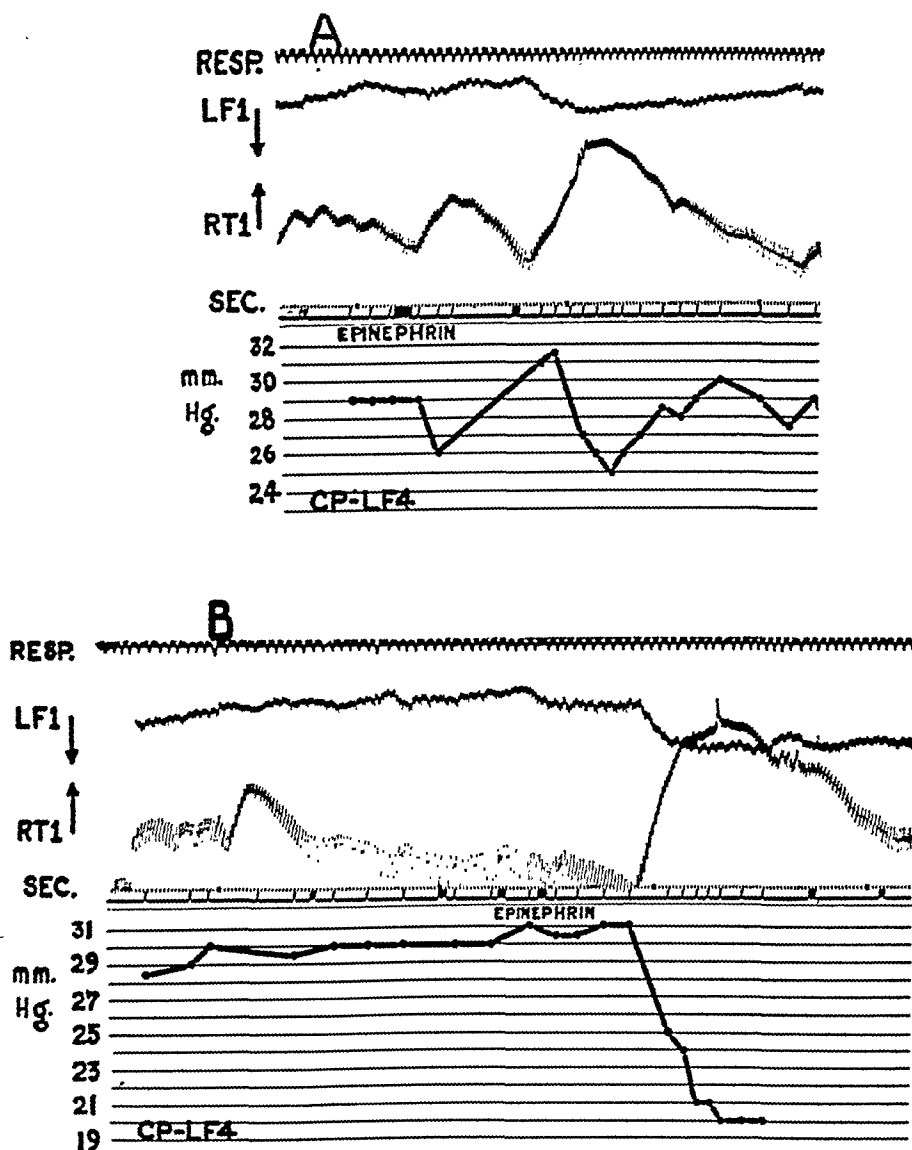


FIG. 4. SUBJECT C. M. RAYNAUD'S DISEASE WITH SCLERODERMA OF HANDS AND FEET

A and B. Simultaneous observations of capillary blood pressure in a sympathectomized digit and of digital volume in a sympathectomized and in a normally innervated digit during vasoconstrictions induced by the intravenous injection of 1 gamma of epinephrin on two occasions. Preganglionic sympathectomy of left upper extremity. Intact innervation right lower extremity. Capillary blood pressure measured in the venous limb of the same capillary loop. Skin temperature LF3, 35.0° C. Room temperature, 30.2° C. Arterial pressure, left arm 112/72 mm. Hg. Note absence in the sympathectomized (LF1) digit of the spontaneously occurring vasoconstrictions recorded in the digit (RT1) with intact innervation.

after interruption of the sympathetic pathways, intravenously administered epinephrin caused reductions in both digital volume and capillary blood pressure of a degree equal to that occurring in normally innervated digits.

Because of technical difficulties, most of the foregoing observations were made on the abnormally large capillaries of patients with Raynaud's disease. General conclusions can, therefore, be reached only with reservations. However, it was reassuring to find that under the conditions imposed, neurogenic vasoconstrictions affected capillary blood pressure similarly in normal and abnormally large capillaries.

The contemplated comparison of capillary blood pressure in normal and hypertensive subjects made it desirable to find a state during which the capillary blood pressure would be relatively constant and relatively unaffected by vasoconstrictor stimuli. It was hoped that reflex vasodilatation or the local hyperemia induced by histamin would provide such a state. This proved not to be the case. Neurogenic vasoconstrictions, with resulting changes in capillary blood pressure, could be abolished only by the interruption of the sympathetic pathways. Even then, epinephrin injected intravenously continued to induce a decrease in both digital volume and capillary blood pressure. However, it is believed that during moderate digital vasodilatation the changes induced in capillary blood pressure by neurogenic vasoconstriction are not so great as to preclude comparative studies.

It is emphasized that the data here presented and the conclusions derived therefrom, apply only to the digital capillaries. The digits possess specialized vascular areas, the blood flow through which is readily affected by many factors. The capillary blood pressure in these specialized areas need not necessarily be indicative of the capillary blood pressure elsewhere in the body. Generalized conclusions based upon data derived from local vascular areas should, therefore, be viewed with reservation.

SUMMARY

1. In the normal-sized digital capillaries of healthy subjects and of hypertensive patients, neurogenic vasoconstrictor stimuli brought about decreases in capillary blood pressure of from 5 per cent to 33 per cent.

2. Reflex vasodilation in the digit, even when combined with local vasodilatation produced by histamine, failed to prevent the fall in capillary blood pressure which occurred in response to neurogenic vasoconstrictor stimuli.

3. The percentage variation in digital capillary blood pressure was considerably smaller than the percentage variation in digital blood flow which has been reported to occur during similarly induced vasoconstrictions.

4. In the abnormally large digital capillaries of patients with Raynaud's disease and scleroderma, neurogenic vasoconstrictions, and vasoconstrictions induced by the intravenous injection of epinephrin, were usually accompanied by decreases in capillary blood pressure.

5. After interruption of the sympathetic nervous pathways to the digits of patients with Raynaud's disease and scleroderma, neurogenic vasoconstrictor stimuli failed to induce in the sympathetomized digits either vasoconstriction or fall in capillary blood pressure. On the other hand, intravenously injected epinephrin continued to cause both vasoconstriction and fall in capillary blood pressures.

6. These observations have been interpreted as indicating (a) that although strong physiologic vasoconstriction mediated through sympathetic nervous pathways may be accompanied by a fall in digital capillary blood pressure, the fall is relatively slight; and (b) that the digital capillary blood pressure may remain at a relatively constant level during wide fluctuations in digital blood flow.

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This investigation was conducted with the technical assistance of Miss Margot Robinson and Miss Sara B. Merritt, B.S.

BIBLIOGRAPHY

1. Wilkins, R. W., Doupe, J., and Newman, H. W., The rate of blood flow in normal fingers. *Clin. Sc.*, 1938, 3, 403.
2. Burton, A. C., The range and variability of the blood flow in the human fingers and the vasomotor regulation of body temperature. *Am. J. Physiol.*, 1939, 127, 437.
3. Landis, E. M., Micro-injection studies of capillary blood pressure in human skin. *Heart*, 1930, 15, 209.

4. Eichna, L. W., and Bordley, J., III, Capillary blood pressure in man. Comparison of direct and indirect methods of measurement. *J. Clin. Invest.*, 1939, 18, 695.
5. Smithwick, R. H., Modified dorsal sympathectomy for vascular spasm (Raynaud's disease) of the upper extremity. A preliminary report. *Ann. Surg.*, 1936, 104, 339.
6. Smithwick, R. H., The value of sympathectomy in the treatment of vascular disease. *New Eng. J. Med.*, 1937, 216, 141.
7. Stürup, G., Bolton, B., Williams, D. J., and Carmichael, E. A., Vasomotor responses in hemiplegic patients. *Brain*, 1935, 58, 456.
8. Bolton, B., Carmichael, E. A., and Stürup, G., Vasoconstriction following deep inspiration. *J. Physiol.*, 1936, 86, 83.
9. Wilkins, R. W., and Eichna, L. W., Blood flow to the forearm and calf. I. Vasomotor reactions: rôle of the sympathetic nervous system. *Bull. Johns Hopkins Hosp.*, 1941, 68, 425.
10. Fahr, G. E., and Ershler, I., Studies of factors concerned in edema formation; hydrostatic pressure in capillaries during edema formation in right heart failure. *Ann. Int. Med.*, 1941, 15, 798.
11. Eichna, L. W., and Bordley, J., III, Capillary blood pressure in man. Direct measurements in the digits of normal and hypertensive subjects during vasoconstrictions and vasodilations variously induced. *J. Clin. Invest.*, 1942, 21, 711.

CAPILLARY BLOOD PRESSURE IN MAN. DIRECT MEASUREMENTS IN THE DIGITS OF NORMAL AND HYPERTENSIVE SUBJECTS DURING VASOCONSTRICTION AND VASODILATATION VARIOUSLY INDUCED^{1, 2}

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The normal cardiac output (1) in essential hypertension implies a normal "total" blood flow to the entire body. The elevated arterial pressure has, therefore, been attributed to an increase in the "total" resistance of the vascular system. The arterioles are considered to contribute the major portion of this increased resistance.

Correlated determinations of arterial and capillary blood pressures in normal and hypertensive subjects should reveal the nature and location of the vascular resistance in hypertension. In hypertensive states, the capillary blood pressure has been measured (2 to 5), but by indirect methods, the reliability of which has been questioned (6). It seemed advisable, therefore, to study the capillary blood pressure of normal and hypertensive subjects by the accurate, direct, micro-injection method (Landis) (7), and to make the observation not only under resting conditions but also during influences known to alter the circulation in the areas under study.

METHODS

The capillary blood pressure was determined in the nail-folds of the fingers. The direct micro-injection method (Landis) (7, 6) was employed throughout.

General. All of the methods, conditions, and precautions, fully described in a previous communication (8) under the *general* category of methods, were rigidly maintained in the present study. This pertained to equipment, surroundings, and subjects.

Particular. Only pressures obtained when the blood flow in the capillaries remained visibly unaltered are recorded as *capillary blood pressures*. At times, capillaries were so pierced that the micropipette obstructed completely the lumen of the capillary. Blood flow

through the capillary ceased. Blood now entered the micropipette from one limb of the capillary and was expelled into the same limb when pressure in the micropipette was raised. The micropipette and capillary-limb acted jointly as a tube leading to the next most adjacent blood channel, arteriole, or venule. Equilibrium between micropipette-pressure and capillary blood pressure under these circumstances was believed to indicate the pressure in the nearest arteriole or venule. Readings obtained under these conditions are termed "arteriole" blood pressure and "venule" blood pressure, respectively.

Each value of capillary blood pressure indicated the pressure in a single capillary, and was obtained by averaging the individual values of a series of readings made during a single continuous observation on that capillary. Single readings which could not be checked by successive readings have been discarded as lacking sufficient reliability. Each value of arteriole or venule blood pressure was obtained in a similar manner.

Observations were made on 3 groups of subjects. (1) Subjects with normal arterial pressures: normal individuals and patients convalescing from illnesses not associated with circulatory disturbances, hereafter termed normal subjects. (2) Patients with elevated arterial pressures: excepting 1 patient with acute nephritis, these subjects had essential hypertension; the majority were of the benign type, several were of the malignant type. (3) Subjects with low arterial pressure, either in association with their systemic illnesses, or as a result of local arterial disease, were included in the normal group. In no patient in any group, was there evidence of congestive heart failure or of an increase in venous pressure.

RESULTS

In both normal and hypertensive subjects, considerable variation in digital capillary blood pressure was found; not only from individual to individual but also in the same subject from day to day and even in adjacent capillaries of the same nail-fold during a single experiment. During moderate vasodilatation of the digital circulation, alterations in capillary blood pressure were occasionally observed during a single series of readings throughout which the micropipette remained in the same location in a given capillary. Similar

¹ Supported by a grant from The Commonwealth Fund for the study of essential hypertension.

² A preliminary report of these observations was read by title at the annual meeting of The American Society for Clinical Investigation, May 5, 1941 (J. Clin. Invest., 1941, 20, 458).

TABLE I

Capillary blood pressure during two series of measurements in the same location of the same capillary during a single experiment

Subject (Sex, age)	Diagnosis	Skin temperature	Arterial pressure	Location where capillary blood pressure was measured	Interval between readings	Capillary blood pressure		
						First series	Second series	Difference
M. E. (F, 30)	Normal	° C. 33.5	mm. Hg 116/78	Summit	minutes 26	mm. Hg 25.5	mm. Hg 33	mm. Hg 7.5
E. S. (F, 28)	Essential hypertension	34.4	164/110	Venous limb	18	18	20	2
		34.4	164/110	Venous limb	13	23	18	5
		33.7	168/110	Venous limb	7	28	38	10
T. R. (F, 38)	Essential hypertension	35.9	156/110	Arteriole	11	46	47	1
V. M. (F, 29)	Essential hypertension	31.1	164/120	Arteriolar limb	23	28	28.5	0.5
F. L. (F, 33)	Essential hypertension	35.3	170/120	Arteriolar limb	15	23	22	1.0
		30.5	166/114	Arteriolar limb	44	10.5*	28*	17.5
H. B. (F, 29)	Essential hypertension	34.0	160/114	Summit	10	30	33	3

Arterial pressure and skin temperature essentially unaltered during the two series of readings.

* In this experiment skin temperature rose from 30.5° C. to 32.2° C. and arterial pressure from 166/114 to 174/120 between the two series of readings.

variations also occurred during reflex vasodilatation, a state considered to be associated with a relatively constant digital circulation. Repiercing the same capillary in the same location, at intervals throughout a single experiment, yielded capillary blood pressures which were usually comparable (Table I). At times large differences were observed between determinations, even when an effort was made to keep the digital circulation constant (Table I).

Effect of increased local venous pressure

A previous study (6) confirmed the observations of Landis (7), that induced increases in local venous pressure were accompanied by rises in capillary blood pressure to values which exceeded the venous pressure.

Including the cases already reported (6), the capillary blood pressure was measured in the same location, in the same capillary, at normal and elevated digital venous pressures, in 22 subjects with varying arterial pressures (Table II). In all subjects, the capillary blood pressure in all lo-

cations of the capillary rose, in most instances to exceed local venous pressure by 1 mm. Hg to 15 mm. Hg. The response of the capillary blood pressure to increases in venous pressure was similar in both hypertensive and normal subjects (Table II).

Effect of vasoconstriction in the digits

Another study (8) showed that neurogenic digital vasoconstrictions were accompanied by decreases in digital capillary blood pressure, which in 4 hypertensive patients were of approximately the same duration and magnitude as in 3 normal subjects (Figure 1). Neither reflex vasodilatation nor the local hyperemia induced by the intradermal injection of histamine acid phosphate (1:100 in salt solution) prevented these falls in capillary blood pressure in normal or hypertensive subjects (8).

Effect of digital skin temperature

No determinations were made of the capillary blood pressure in the same capillary during sig-

nificant changes in digital skin temperature. However, the temperature of the digital pad was determined at the time of each capillary blood pressure measurement. Therefore, some correlation of these two factors is possible (Figures 2 and 3).

With the hands moderately warm, capillary blood pressure seemed unaffected by digital skin temperature. At all skin temperatures between 27° C. and 35° C., the capillary blood pressures in all locations of the capillary scattered widely, and approximately equally, in both normal (Figure 2) and hypertensive (Figure 3) subjects. In both groups of subjects, the degree of variation was much the same at the lower as at the higher temperatures within this range. At the warmer

skin temperatures, slightly higher capillary blood pressures were obtained in both the arteriolar and venous limbs of the hypertensive patients than in the normal subjects. These differences were not definite. Only at very low skin temperatures did the digital capillary blood pressure fall below the limits obtained during moderate digital vasodilatation. For example, in one hypertensive subject, the capillary blood pressure in the arteriolar limb fell to 7.5 mm. Hg when the digital skin temperature was 24.8° C. Blood flow through the capillary at this time was abnormally slow.

Gradient of fall of pressure in the capillary

The capillary blood pressure was determined in 60 subjects with normal arterial pressures, in 46

TABLE II

The effect of locally increased venous pressure upon the blood pressure in the same capillary

Subject (Sex, age)	Diagnosis	Arterial pressure	Skin temperature	Location in capillary where pressure was measured	Capillary blood pressure		Cuff pressure*	Difference be- tween capillary blood pressure and cuff pressure during venous congestion
					Initial	During venous congestion		
I. B. (F, 19)	Normal	mm. Hg 116/72	° C. 31.1	Arteriolar limb	mm. Hg 19	mm. Hg 54 to 60	mm. Hg 44	mm. Hg +10
B. B. (F, 13)	Diabetes mellitus	92/62	29.0	Venous limb	12	51.5	53 (51.8)	- 1.5
		92/62	31.1	Venous limb	13	51.5	52	- 0.5
		92/62	31.1	Venous limb	19	61.5	51	+10.5
H. B. (M, 15)	Convalescent rheumatic heart disease	116/76	28.1	Arteriolar limb	22	58.5	52 (46.5)	+ 6.5
H. B. (F, 25)	Syphilis	80/50	31.1	Venous limb	11.5	46	44 (42.8)	+ 2.0
H. G. (F, 26)	Normal	112/82	29.7	Summit	53	62.5	61	+ 1.5
C. J. (M, 47)	Normal	112/82	29.7	Arteriolar limb	20	76	80	- 4.0
		112/82	29.7	Arteriolar limb		60	59	+ 1.0
B. M. (F, 31)	Convalescent otitis media	106/76	29.6	Arteriolar limb	35	69.5	60 (57.8)	+ 9.5
		106/76	29.8	Venous limb	35.5	68	60 (57.8)	+ 8.0
G. M. (M, 19)	Normal	126/74	27.1	Venous limb	14	39	36	+ 3.0
		116/76	28.9	Venous limb	11	49.5	46	+ 3.5
W. O. (M, 25)	Normal	124/66	30.5	Venous limb	13	49	50	- 1.0
E. R. (M, 21)	Normal	130/76	30.0	Arteriolar limb	41	66	52	+14
		130/76	30.0	Arteriolar limb	36	68	52	+16
J. W. (M, 32)	Convalescent pneumonia	104/60	28.9	Venous limb	23.5	54	54 (47.9)	0.0
H. G. (F, 41)	Raynaud's disease	110/74	27.3	Arteriolar limb	12	41	40	+ 1.0
		110/74	27.3	Summit	13	44	43	+ 1.0

TABLE II—Continued

Subject (Sex, age)	Diagnosis	Arterial pressure	Skin temperature	Location in capillary where pressure was measured	Capillary blood pressure		Cuff pressure*	Difference be- tween capillary blood pressure and cuff pressure during venous congestion
					Initial	During venous congestion		
M. L. (F, 48)	Raynaud's disease with scleroderma	<i>mm. Hg</i>	<i>° C.</i>		<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>
		110/72	26.5	Venous limb	18	60	60 (57.9)	0.0
		108/62	23.8	Venous limb	20	67.5	63	+ 4.5
		110/72	33.0	Arteriolar limb	20	64	60	+ 4.0
		110/72	33.0	Venous limb	19	62	61	+ 1.0
F. G. (M, 31)	Raynaud's disease	130/76	25.7	Venous limb	16.5	52	52 (47.1)	0.0
			28.1	Venous limb	35	73	63	+10.0
		130/76	28.1	Venous limb	22	66	61	+ 5.0
		118/66	30.3	Venous limb	20	53.5	54	- 0.5
		118/66	30.3	Venous limb	14	38.5	43	- 4.5
C. B. (M, 35)	Hypertension	222/148	35.0	Summit	52	128	82	+46.0
J. B. (M, 41)	? Hypertension	148/90	28.6	Venous limb	15	50	50	0.0
H. B. (F, 29)	Hypertension	166/130	27.7	Venous limb	14	66	63	+ 3.0
W. E. (M, 50)	Hypertension	200/118	29.0	Venous limb	29	58	52	+ 6.0
H. F. (F, 36)	Hypertension	220/140	30.2	Venous limb	18	52	52	0.0
F. L. (F, 33)	Hypertension	174/114	32.7	Venous limb	12	63	60	+ 3.0
		174/114	32.7	Venous limb	18	83	80	+ 3.0
		192/128	31.5	Venous limb	22	95	90	+ 5.0
		192/128	31.5	Venous limb	not deter- mined	102	90	+12.0
N. P. (F, 23)	Hypertension	174/110	31.5	Venous limb	14	77	80	- 3.0
J. P. (M, 36)	Hypertension	236/146	30.3	Arteriolar limb	49	82	81	+ 1.0

* Local venous pressure usually fell several mm. Hg below cuff pressure. Figures in parentheses give the actually determined venous pressure at the cuff pressure indicated by the immediately preceding figure.

patients with essential hypertension, and in one patient with acute nephritis. In the "normal" group, the arterial pressure averaged 119/74 (mean pressure 96 mm. Hg). Systolic pressure ranged from 80 mm. Hg to 156 mm. Hg, and diastolic pressure, from 50 mm. Hg to 98 mm. Hg. In the hypertensive group, the average arterial pressure was 188/124 (mean pressure 156 mm. Hg); the systolic pressure varied from 132 mm. Hg to 254 mm. Hg, and the diastolic pressure, from 96 mm. Hg to 186 mm. Hg. In one subject with multiple aneurysms, the arterial pressure was unobtainable.

In both groups of subjects, the wide variations in the capillary blood pressure for the same loca-

tion in the capillary led to considerable overlapping of the values for arteriolar and venous limbs. Nevertheless, the values for corresponding locations in the capillary were approximately the same in both the hypertensive and normal subjects (Table III). The scattering and overlapping were less marked when determinations were made during a single experiment on adjacent capillaries. At such times, the values for the two limbs of the capillaries were both in the same range, with the arteriolar limb pressure usually exceeding slightly the venous limb pressure.

Assuming the average values for each location in the capillary to be representative of the capil-

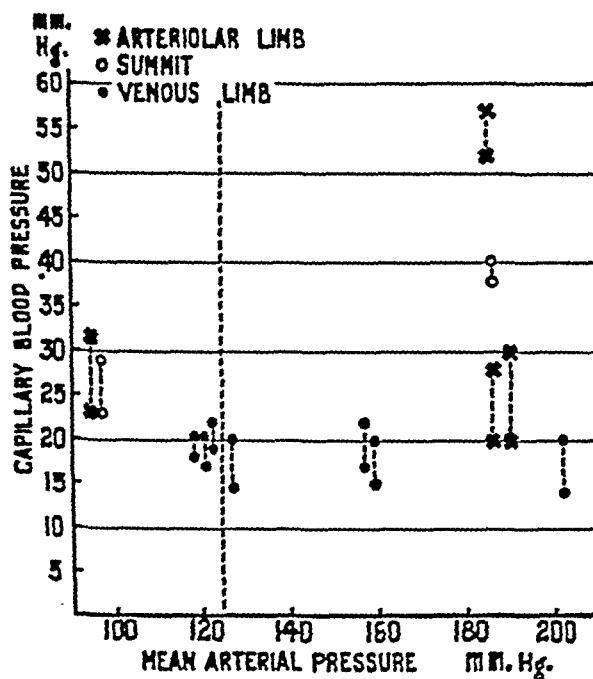


FIG. 1. CHANGES IN CAPILLARY BLOOD PRESSURE IN THE SAME CAPILLARY IN NORMAL AND HYPERTENSIVE SUBJECTS FOLLOWING NEUROGENIC VASOCONSTRICTOR STIMULI

Following the stimuli the pressures always fell, and approximately equally in both groups of subjects.

Construction of charts

All charts. Ordinates; always capillary blood pressure in mm. Hg. Abscissae; usually mean arterial pressure in mm. Hg, except Figures 2, 3 (skin temperature °C.). The long, vertical, dotted line indicates the upper limit of normal mean arterial pressure.

Scatter charts. (Figures 2, 3, 4, 6, 7, 9.) Each dot represents the average digital capillary blood pressure in the designated location in a single capillary at the mean arterial pressure or skin temperature indicated by the abscissa. Each heavy horizontal arrow and number above the abscissa line indicates the average of all of the pressures represented by the corresponding dots.

Charts of changes in single capillaries. (Figures 1, 8.) Digital capillary blood pressures in the same location of the same capillary are connected by vertical lines. When the lines are solid, the pressure rose from the lower to the higher value; when dotted, the pressure fell from the higher to the lower value.

lary blood pressure in that location for the group, then the gradient of fall of pressure from arteriolar limb to venous limb was 8.7 mm. Hg (30.6 mm. Hg to 21.9 mm. Hg) in the "normal" subjects, and 13.1 mm. Hg (35.9 mm. Hg to 22.8 mm. Hg) in the hypertensive subjects. An increase

of 50.5 per cent in the hypertensive subjects. But, when the gradient of pressure was measured in the same capillary, no significant difference was found in the gradient between corresponding locations of the capillary in 9 normal and 12 hypertensive subjects (Table IV).

In both normal and hypertensive subjects, the gradient of fall of pressure from "arteriole" to "venule" was more marked than the gradient from arteriolar limb to venous limb of the capillary (Figure 4).

The average arteriole-venule gradient for the normal subjects was 38.1 mm. Hg, for the hypertensive subjects 34.9 mm. Hg. In a few instances, both arteriole and venule blood pressure in the hypertensive subjects exceeded the highest values obtained in normal subjects. There was no correlation between arteriole or venule blood pressure and the mean arterial pressure.

Digital capillary blood pressure during procedures designed to dilate the arterioles

Reflex vasodilatation. Although reflex vasodilatation is associated with a constant, reproducible, and maximal digital skin temperature, digital capillary blood pressure determined during this state still varied widely in both normal and hypertensive subjects (Figure 5). Even during a single experiment, throughout which digital skin temperature was maintained at the maximum level, considerable variation persisted in the digital capillary blood pressures measured in the same location of adjacent capillaries of a given individual. The limits of this variation and the average capillary blood pressures for both arteriolar and venous limbs were practically the same in the few hypertensive and normal subjects studied (Figure 5). Moreover, the range of variation during maximal reflex vasodilatation approximated that obtained during moderate digital vasodilatation.

During maximal reflex vasodilatation the average capillary blood pressure in the venous limb exceeded values obtained during the resting state, but the average pressure in the arteriolar limb remained unchanged (Figures 5, 2, and 3). As a result, the gradient of pressure from arteriolar limb to venous limb of the capillary became less steep: 2 mm. Hg in the normal subjects, practically zero in the hypertensive subjects. It is

known that the digital blood flow during this state is, nevertheless, much increased.

Reactive hyperemia. The digital capillary blood pressure was always determined in the same location in the same capillary during reactive hyperemia as it had been during the resting state. With circulatory arrest, the blood continued to flow for 5 to 10 seconds, then stopped. It gradually became dark blue, more compact and seemingly more viscid, as indicated by its more sluggish movement in, and adherence to, the micropipette tip. Apparently, the red blood cells packed together, presumably as a result of the loss of plasma fluid. In the summits of 2 capillaries of a patient with Raynaud's disease, the digital capillary blood pressure at this time measured 11 mm. Hg and 13 mm. Hg.

On release of the circulation, the mass of packed red blood cells tended to adhere to the wall of the capillary and, at times, several seconds elapsed

before the mass was pushed onward and blood flow became reestablished. Sticking of the erythrocytes to the wall of the capillary was observed most frequently in those capillaries which had been previously pierced several times. Occasionally, it was necessary to prod the tissue about the capillary with the micropipette before the red blood cells moved forward and swift blood flow returned to the capillary. At times, even this procedure did not suffice, and the capillary remained "permanently" in stasis. During reactive hyperemia, the capillaries appeared very pink but not particularly dilated.

The above findings were similar in normal and hypertensive subjects.

In the few studies on the normal sized capillaries of normal and hypertensive subjects, digital capillary blood pressure during reactive hyperemia was essentially the same as during the resting state (Table V). The differences between pressures

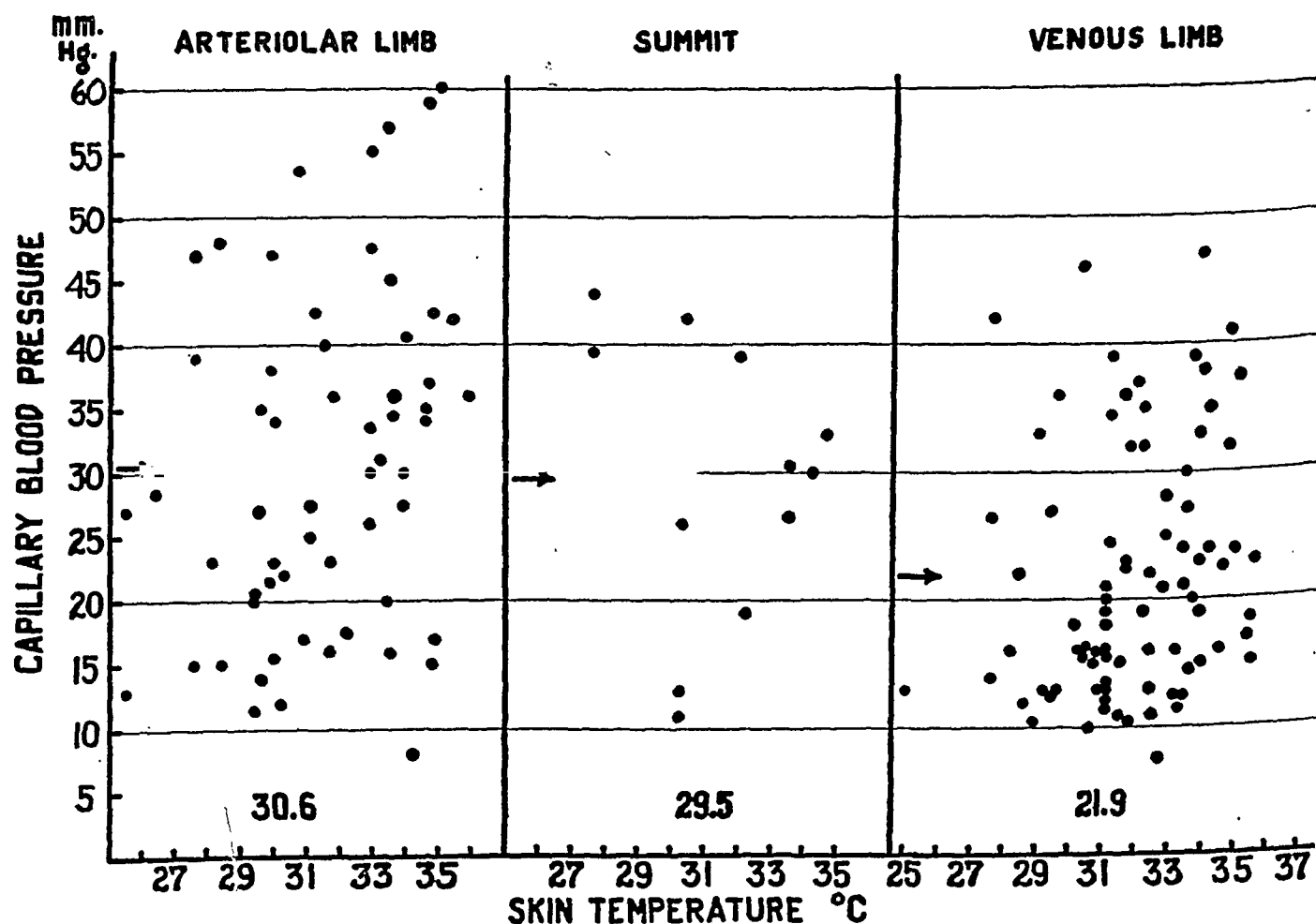


FIG. 2. DIGITAL CAPILLARY BLOOD PRESSURE IN DIFFERENT LOCATIONS IN THE CAPILLARY AT VARYING LEVELS OF DIGITAL SKIN TEMPERATURE. SUBJECTS WITH NORMAL ARTERIAL PRESSURE

The range of pressures is the same at both low and high skin temperatures.

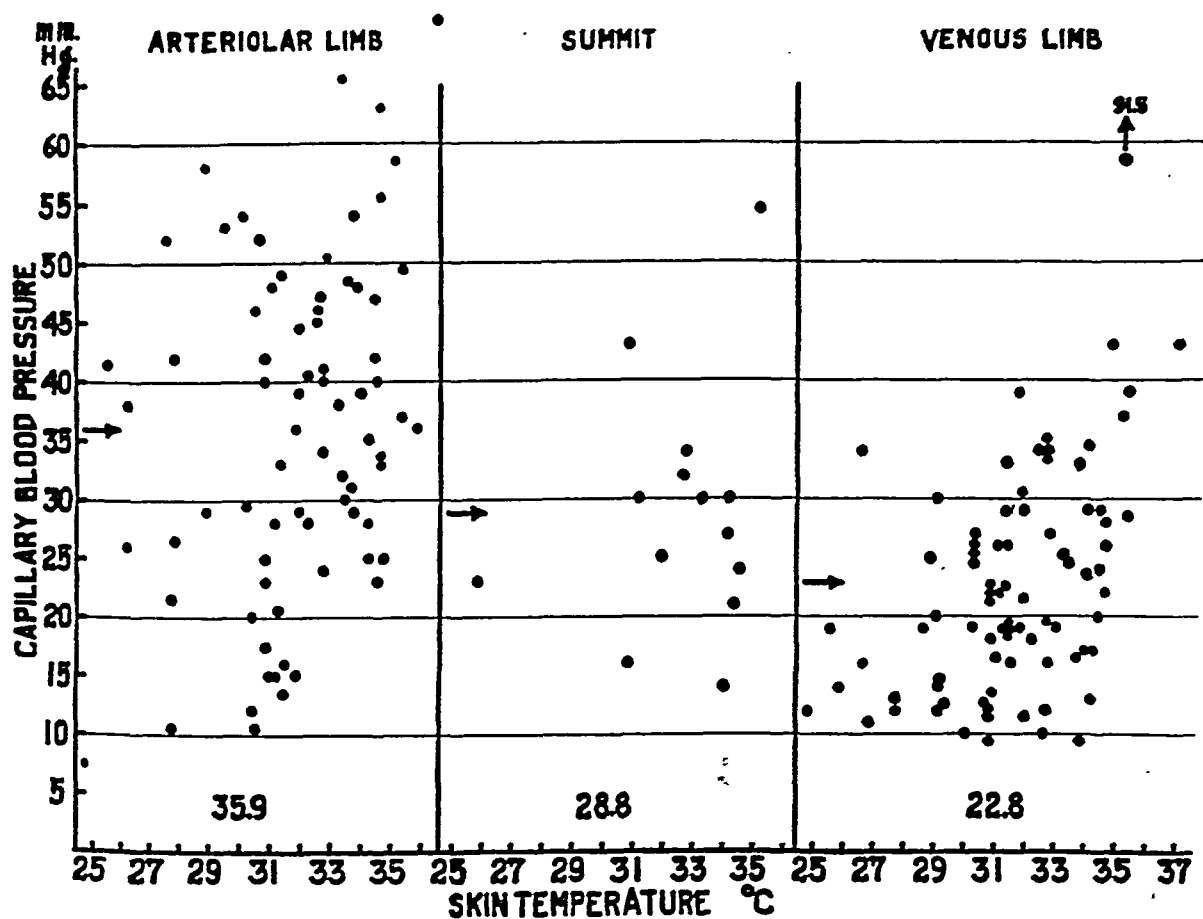


FIG. 3. DIGITAL CAPILLARY BLOOD PRESSURE IN DIFFERENT LOCATIONS IN THE CAPILLARY AT VARYING LEVELS OF DIGITAL SKIN TEMPERATURE. HYPERTENSIVE SUBJECTS

The pressures vary over the same range at both low and high temperatures.

obtained during the two states did not exceed — 4 mm. Hg. to + 1.5 mm. Hg (Table V).

Difficulty in piercing quickly normal sized capillaries during the early period of reactive hyperemia (transitory at best) led to studies on the more easily pierced abnormally large digital capillaries of patients with Raynaud's disease.

In the summits and venous limbs of 5 capillaries in 2 subjects with Raynaud's disease, the capillary blood pressure during reactive hyperemia differed from the resting values by only — 1.5 mm. Hg to + 5.5 mm. Hg (average 2.5 mm. Hg), usually in the positive direction (Table VI). This maintained whether the reactive hyperemia involved the entire forearm or the digit alone.

After preganglionic sympathectomy of the upper extremity of the same 2 subjects, the observations were repeated with generally similar results (Ta-

ble VII). In the summits and venous limbs of 10 capillaries, the capillary blood pressure during reactive hyperemia differed from the resting values by — 11.0 mm. Hg to + 4.5 mm. Hg (average 4 mm. Hg) usually in a negative direction (Table VII). The occasional considerable difference was obtained when the determination was made quickly after onset of the hyperemia, when the preceding ischemia was of long duration (10 minutes rather than 5), and when the area of hyperemia was great (forearm rather than digit alone).

Histamine acid phosphate (1:100) injected intradermally

These experiments were few in number. During the erythema produced by the intradermal injection of histamine, considerable variation in digital capillary blood pressure still persisted in both

TABLE III
Capillary blood pressure in normal and hypertensive subjects

	Arteriolar limb				Summit				Venous limb			
	Number of subjects	Number of capillaries	Capillary pressure		Number of subjects	Number of capillaries	Capillary pressure		Number of subjects	Number of capillaries	Capillary pressure	
			Range	Average			Range	Average			Range	Average
Normal	31	59	mm. Hg 7.5 to 60	mm. Hg 30.6	12	14	mm. Hg 11 to 44	mm. Hg 29.5	42	84	mm. Hg 7.5 to 47	mm. Hg 21.9
Hypertensive	29	75	7.5 to 70.5	35.9	10	14	14 to 54.5	28.8	35	83	9.5 to 43	22.8

Most of the determinations were made at digital skin temperature between 30° C. and 35° C.

the arteriolar and venous limbs of normal and hypertensive subjects (Figure 6). In both groups, and for both locations in the capillary, the values did not exceed the upper limits obtained during moderate digital vasodilatation. However, the average capillary blood pressures in all locations of the capillary rose in both groups of subjects to exceed the average values obtained during resting conditions (Figure 6). These in-

creases were greater in the venous limbs than in the arteriolar limbs. In the normal subjects, the average capillary blood pressure in the venous limb rose 8.8 mm. Hg, and in the arteriolar limb only 3.6 mm. Hg. In the hypertensive subjects, the increase in average capillary blood pressure in the venous limb was 18.7 mm. Hg, and in the arteriolar limb, 10.7 mm. Hg. Because of the greater increases in hypertensive subjects, the re-

TABLE IV
Gradient of blood pressure determined in the same capillary during a single experiment

Subject (Sex, age)	Diagnosis	Skin tempera- ture	Arterial pressure	Capillary blood pressure					Gradient
				Arteriole	Arteriolar limb	Summit	Venous limb	Venule	
SUBJECTS WITH NORMAL ARTERIAL PRESSURE									
E. W. (M, 22)	Tetralogy of Fallot	° C. 29.9	mm. Hg 118/74	mm. Hg 33	mm. Hg 21.5	mm. Hg	mm. Hg	mm. Hg	mm. Hg 11.5
A. G. (M, 18)	Normal	30.2	130/72		12	11			1
R. H. (M, 26)	Convalescent pneumonia	32.9	144/56		26	25			1
S. B. (M, 37)	C. N. S. syphilis	33.6	112/64		34.5	22			12.5
C. J. (F, 37)	Normal	31.1	148/96		25		20		5
C. J. (M, 47)	Clubbed fingers	29.4	114/82		20		27		-7
W. H. (M, 33)	Convalescent pneumonia	33.6	120/78		36		14		22
F. K. (F, 16)	Convalescent nephritis	33.4	138/98		57		24		33
J. A. (M, 41)		32.4	126/96?				13	12.5	0.5

TABLE IV—Continued

Subject (Sex, age)	Diagnosis	Skin tempera- ture	Arterial pressure	Capillary blood pressure					Gradient
				Arteriole	Arteriolar limb	Summit	Venous limb	Venule	
HYPERTENSIVE SUBJECTS									
E. B. (F, 35)	Malignant hypertension	° C. 34.4	mm. Hg 230/170	mm. Hg 58	mm. Hg 47				mm. Hg 11
T. R. (F, 38)	Essential hypertension	33.8	172/130	5	11				-6
E. S. (F, 28)	Essential hypertension	34.6	182/118	74	33.5				40.5
C. B. (M, 35)	Essential hypertension	35.1	222/148	76		54.5			21.5
H. B. (M, 35)	Essential hypertension	34.0	174/130	41			34.5		6.5
M. K. (F, 50)	Hypertension telangectases	30.8	186/96		25	16			9
V. M. (F, 29)	Essential hypertension	31.1	164/120		28	30			-2
E. S. (F, 28)	Essential hypertension	34.2	166/112		25	39			-14
J. B. (M, 41)	Subarachnoid hemorrhage	31.4	182/106		16	20.5			-4.5
H. B. (F, 29)	Essential hypertension	35.3	154/112		37		28.5		8.5
H. B. (F, 29)	Essential hypertension	34.6	152/106		33		26		7
E. B. (F, 35)	Malignant hypertension	32.8	216/160		50.5		27		23.5
F. L. (F, 33)	Essential hypertension	32.7	174/116		41		12		29
F. L. (F, 33)	Essential hypertension	32.7	174/116		34		16		18
V. M. (F, 29)	Essential hypertension	?	196/124		17.5		9.5		8
C. W. (M, 29)	Essential hypertension	30.2	156/106		29		19		10
E. S. (F, 28)	Essential hypertension	34.6	182/118		33.5		28		5.5
F. L. (F, 33)	Essential hypertension	34.5	170/120			24	22		2
V. M. (F, 29)	Essential hypertension	31.1	164/120			30	26		4
R. E. (M, 37)	Essential hypertension	33.9	204/142				16	14	2
V. M. (F, 29)	Essential hypertension	24.8	154/106				12	11.5	0.5

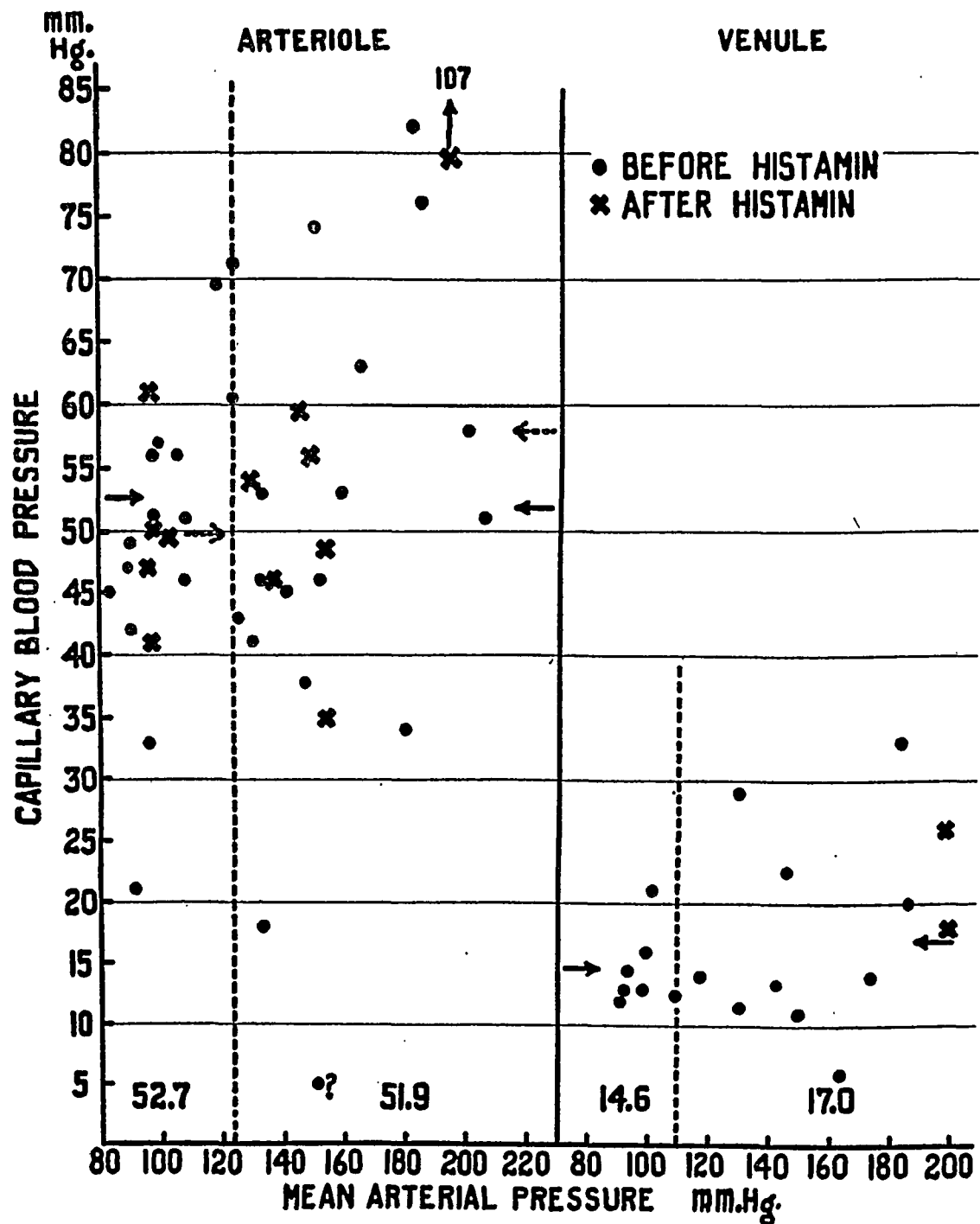


FIG. 4. THE BLOOD PRESSURE IN "ARTERIOLES" AND "VENULES" DURING THE RESTING STATE AND DURING HISTAMINE FLARE IN NORMAL AND HYPERTENSIVE SUBJECTS

The solid horizontal arrows and the numbers above the abscissa line indicate average pressures during the resting state; the dotted arrows, average values during histamine flare. "Arteriole" and "venule" pressures in normal and hypertensive subjects vary over the same range, both during the resting state and during local hyperemia from histamine.

	"Arterioles"				"Venules"			
	Number of subjects	Number of capillaries	Pressure		Number of subjects	Number of capillaries	Pressure	
			Range	Average			Range	Average
Normal.....	11	15	mm. Hg 21 to 71	mm. Hg 52.7	7	7	mm. Hg 12 to 21	mm. Hg 14.6
Hypertensive.....	13	17	18 to 82	51.9	9	10	6 to 33	17.0

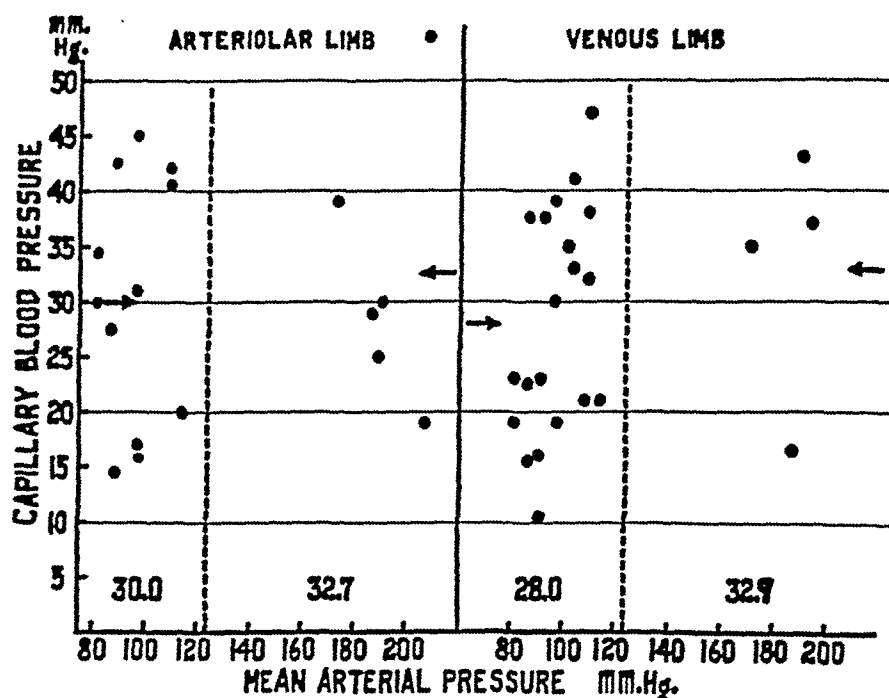


FIG. 5. DIGITAL CAPILLARY BLOOD PRESSURE MEASURED DURING REFLEX VASODILATATION IN NORMAL AND HYPERTENSIVE SUBJECTS

Capillary blood pressure in normal subjects:

12 arteriolar limbs of 7 subjects—14.5 mm. Hg to 45 mm. Hg, average 30 mm. Hg

20 venous limbs of 9 subjects—10.5 mm. Hg to 47 mm. Hg, average 28 mm. Hg

Capillary blood pressure in hypertensive subjects:

6 arteriolar limbs of 5 subjects—19 mm. Hg to 54 mm. Hg, average 32.7 mm. Hg

4 venous limbs of 3 subjects—16.5 mm. Hg to 43 mm. Hg, average 32.9 mm. Hg

In both normal and hypertensive subjects the pressures are similar in both limbs of the capillary.

TABLE V

The effect of reactive hyperemia on the capillary blood pressure. Normal sized capillaries. Innervation intact

Subject (Sex, age)	Diagnosis	Resting state				Reactive hyperemia						Location in capillary where blood pressure was determined
		Skin tem- pera- ture	Arterial pressure		Capillary blood pressure	Skin tem- pera- ture	Arterial pressure		Capillary blood pressure		Duration of and area of ischemia preceding the hyperemia	
			Reading	"Mean"			Reading	"Mean"	Reading	Time of reading after on- set of hyperemia		
H. B. (F, 31)	Essential hypertension	° C. 34.1	mm. Hg 166/116	mm. Hg 141	mm. Hg 29.5	° C. 34.0	mm. Hg 160/114	mm. Hg 137	mm. Hg 31	seconds 23	5 minutes, digit	Summit
F. L. (F, 35)	Essential hypertension	35.0	166/122	144	23	35.0	166/122	144	19	32	5 minutes, digit	Arteriolar limb
E. S. (F, 30)	Essential hypertension	35.0	164/110	137	22.5	33.7 34.1	164/110 168/112	137 140	23 21	24 24	5 minutes, digit 5 minutes, digit	Venous limb Venous limb
M. E. (F, 32)	Normal	33.5	116/78	97	26.5	32.7	118/80	99	22.5 to 25.0	55	5 minutes, digit	Summit

TABLE VI

The effect of reactive hyperemia on the capillary blood pressure. Abnormally large capillaries. Innervation intact

Sub- ject (Sex, age)	Diagnosis	Resting state				Reactive hyperemia						Location in capillary where blood pressure was determined
		Skin tem- pera- ture	Arterial pressure		Capil- lary blood pres- sure	Skin tem- pera- ture	Arterial pressure		Capillary blood pressure		Duration of and area of ischemia preceding the hyperemia	
			Reading	"Mean"			Reading	"Mean"	Reading	Time of reading after on- set of hyperemia		
C. M. (F, 29)	Raynaud's disease	° C.	mm. Hg	mm. Hg	mm. Hg	° C.	mm. Hg	mm. Hg	mm. Hg	seconds		
		26.5	132/88	110	22.5	28.6	132/88	110	24	35	5 minutes, digit	Venous limb
						30.0	132/88	110	26	30	5 minutes, forearm	Venous limb
		32.7	120/80	100	28	30.3	120/80	100	33	45	5 minutes, digit	Summit
		33.6	120/86	103	30.5	33.6	120/86	103	36	30	5 minutes, digit	Summit
						32.8	120/82	101	28	25	5 minutes, forearm	Summit
						32.2	126/82	104	32	25	5 minutes, digit	
F. G. (M, 32)	Raynaud's disease	31.1	120/82	101	19.5	32.6	130/84	107	20	20	5 minutes, forearm	Summit, venous limb (different locations)
		32.3	125/86	106	19.5	32.3	125/86	106	21.5	24	5 minutes, forearm	Venous limb

sultant average capillary blood pressures in both the arteriolar and venous limbs were higher in hypertensive than in normal subjects: arteriolar limb—normal 34.2 mm. Hg, hypertensive 46.6 mm. Hg; venous limb—normal 30.7 mm. Hg, hypertensive 41.5 mm. Hg.

Due to the disproportionately greater increases in venous limb pressure than in the arteriolar limb pressure, the gradient of fall of pressure from arteriolar limb to venous limb was less steep during the erythema produced by histamine than

during the usual moderate digital vasodilatation. In the few normal subjects studied, this gradient was 3.5 mm. Hg and in the hypertensive subjects, 5.1 mm. Hg (Figure 6). The local blood flow during this erythema is believed to be much increased. During the histamine flare, the arteriole blood pressure in both normal and hypertensive subjects was not significantly altered from that during moderate digital vasodilatation alone (Figure 4). In the normal subjects, the average arteriole blood pressure during the resting state was

TABLE VII

The effect of reactive hyperemia on the capillary blood pressure. Abnormally large capillaries. Sympallectomized extremity

Sub- ject (Sex, age)	Diagnosis	Resting state				Reactive hyperemia						Location in capillary where blood pressure was determined
		Skin tem- pera- ture	Arterial pressure		Capil- lary blood pres- sure	Skin tem- pera- ture	Arterial pressure		Capillary blood pressure		Duration of and area of ischemia preceding the hyperemia	
			Reading	"Mean"			Reading	"Mean"	Reading	Time of reading after onset of hyperemia		
C. M. (F, 29)	Raynaud's disease (post sympa- thectomy)	° C.	mm. Hg	mm. Hg	mm. Hg	° C.	mm. Hg	mm. Hg	mm. Hg	seconds		
		30.7	114/68	91	27	30.7	114/68	91	19	35	5 minutes, digit	Summit—flow seemed more slow
		32.3	116/68	92	22.5	32.6	116/68	92	27	35	5 minutes, digit	
		32.4	126/86	106	29.5	32.4	126/86	106	22.5	17	5 minutes, digit	Venous limb
						32.7	128/86	107	24.5	27	5 minutes, forearm	
						33.8	122/82	102	19	15	10 minutes, digit	

52.7 mm. Hg, during the erythema 49.9 mm. Hg, and in the hypertensive subjects, 51.9 mm. Hg and 58 mm. Hg, respectively.

During a single experiment, the capillary blood pressure in the same location in a given capillary was measured, both before and after the production of a histamine-flare. In 3 normal subjects and 6 hypertensive patients, approximately equal changes were induced in the capillary blood pressure by the histamine-flare (Figure 7). No correlation existed between the extent of the change in capillary blood pressure and the mean arterial pressure.

Relation of capillary blood pressure to mean brachial arterial pressure

Neither in normal nor in hypertensive subjects was there any correlation between the digital capillary blood pressure and the systolic, diastolic, or mean arterial pressures. This maintained for the range as well as for average values, and for pressures in both the arteriolar (Figure 8) and venous limbs (Figure 9). The lack of correlation was emphasized by the finding of almost equal digital capillary blood pressures in 2 patients. In one, the brachial arterial pressure was unobtainable (aneurysm), in the other the mean arterial pressure was

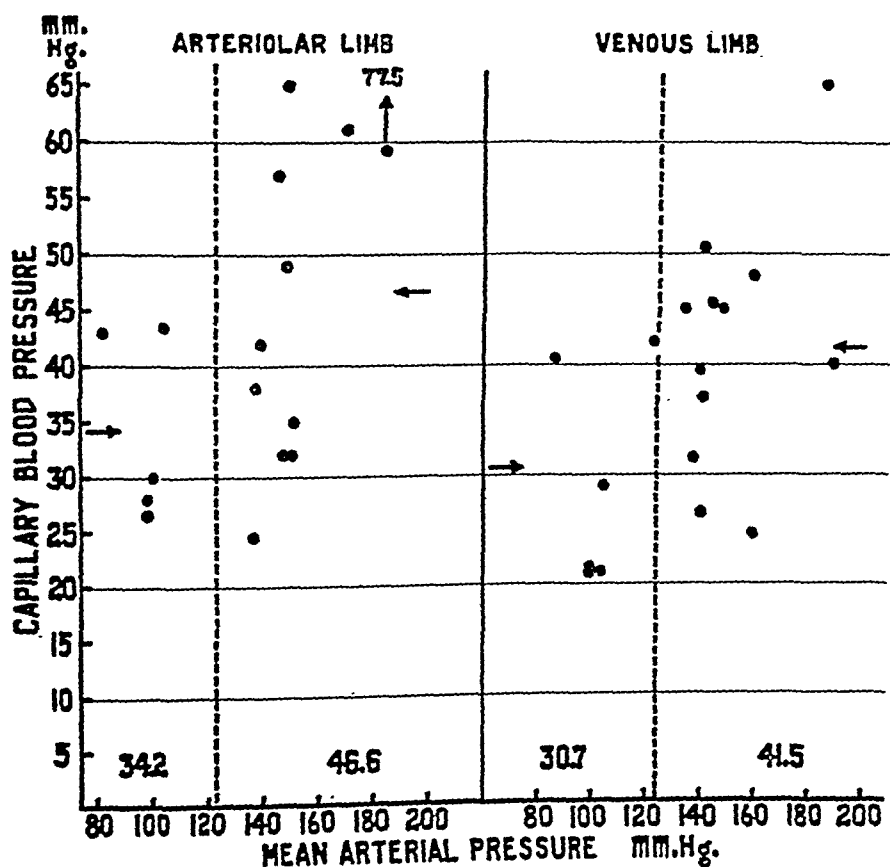


FIG. 6. DIGITAL CAPILLARY BLOOD PRESSURE MEASURED DURING LOCAL HYPEREMIA OF HISTAMINE IN NORMAL AND HYPERTENSIVE SUBJECTS

Capillary blood pressure in normal subjects:

5 arteriolar limbs of 4 subjects—26.5 mm. Hg to 43.5 mm. Hg, average 34.2 mm. Hg

5 venous limbs of 5 subjects—11 mm. Hg to 42 mm. Hg, average 30.7 mm. Hg

Capillary blood pressure in hypertensive subjects:

11 arteriolar limbs of 7 subjects—24.5 mm. Hg to 77.5 mm. Hg, average 46.6 mm. Hg

12 venous limbs of 6 subjects—24.5 mm. Hg to 65 mm. Hg, average 41.5 mm. Hg

In both arteriolar and venous limbs capillary blood pressure is higher in hypertensive than in normal subjects.

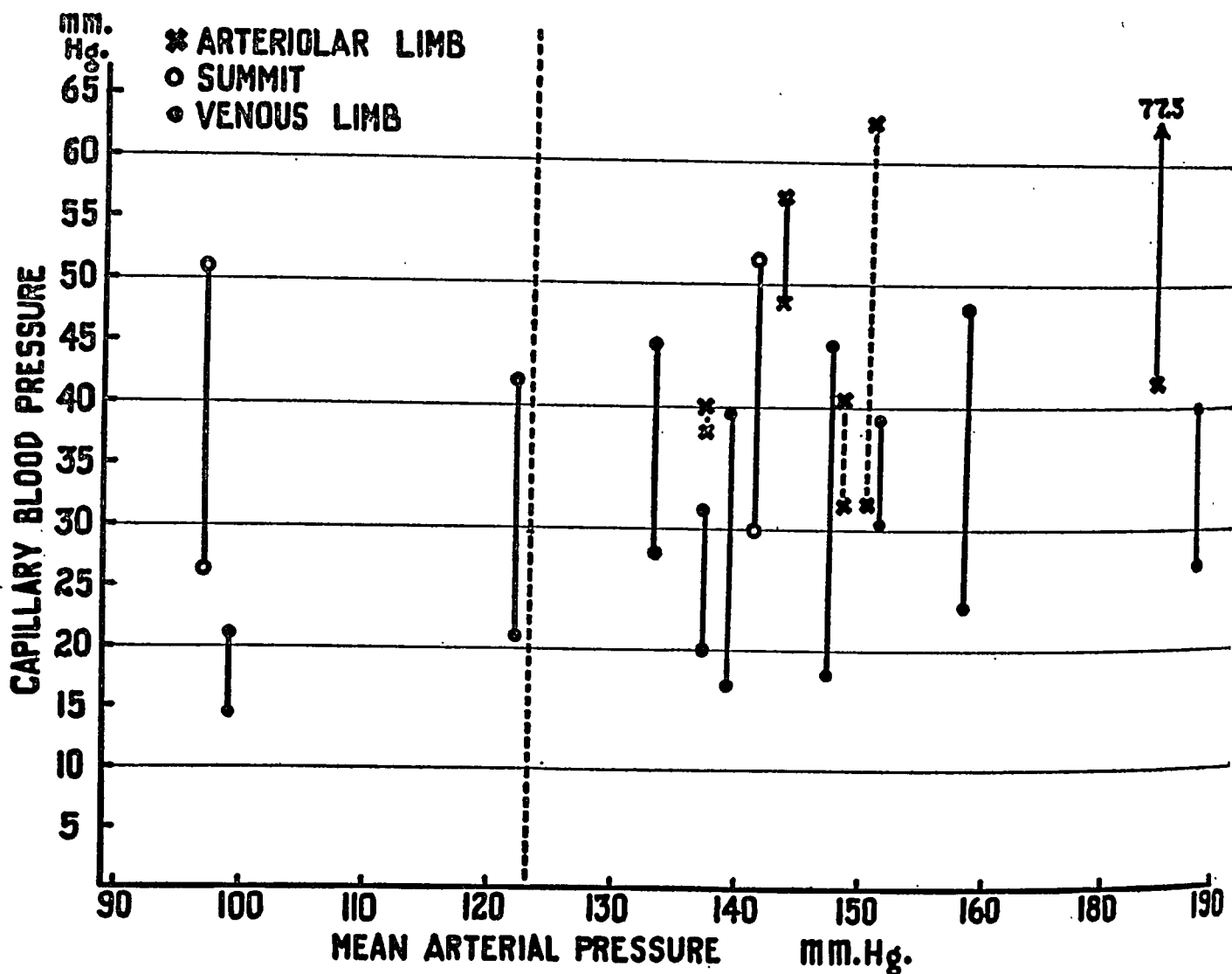


FIG. 7. DIGITAL CAPILLARY BLOOD PRESSURE MEASURED IN THE SAME CAPILLARY BEFORE AND DURING A LOCAL HISTAMINE FLARE IN NORMAL AND HYPERTENSIVE SUBJECTS

Increases in pressure in corresponding locations of the capillary seem as great in normal as in hypertensive subjects.

201 mm. Hg. Lack of correlation between digital capillary blood pressure and arterial pressure persisted during the action of the above described digital vasoconstrictions and vasodilatations.

In 4 hypertensive patients, no significant differences were noted between the capillary blood pressures determined during the hypertensive state and those obtained when the arterial pressure had returned to normal (Table VIII). Reduction in arterial pressure occurred spontaneously in 2 patients, accompanied unilateral nephrectomy in one, and in the fourth, followed bilateral subdiaphragmatic splanchnicectomy and lumbar ganglionectomy (9). Although these determinations of capillary blood pressure were not made in the same capillary, nor in the same location in the capillaries at the two levels of arterial pressure, the

conditions were otherwise kept as nearly similar as possible.

While under observation, a moderately hypertensive patient developed local arterial disease which obliterated the arterial pulsations in the left upper extremity, thereby making indirect determinations of arterial pressure impossible. In the contralateral right arm, the arterial pressure remained moderately elevated. With the circulation to each upper extremity in moderate vasodilatation, the digital capillary blood pressure (determined largely in venous limbs) was almost identical on the two sides (Table VIII).

DISCUSSION

These observations were made on capillaries in the nail-folds of the fingers. The conclusions de-

rived therefrom are limited to this single area and are not to be applied to capillaries in other regions of the body or to the circulation as a whole. In other areas, there may not be a duplication of the peculiarities of anatomic structure (10) (arteriole-venule anastomoses), or of labile physiologic reactivity found in the vascular system of the digits. To draw generalized conclusions from data obtained in this single, specialized area does not seem justifiable. It is to be understood that the following discussion pertains to one area alone, the nail-folds of the fingers.

In both normal and hypertensive subjects, there was a wide scattering and overlapping of the capillary blood pressures for each location in the capillary. This persisted even during procedures (*e.g.*, reflex vasodilatation) designed to produce a standard, reproducible, state of the digital circulation. It is, therefore, difficult to justify general concepts based on average values. The data does, however, indicate the trend of digital capillary blood pressure under a variety of influences.

The digital capillary blood pressure in all locations in the capillary was both qualitatively and

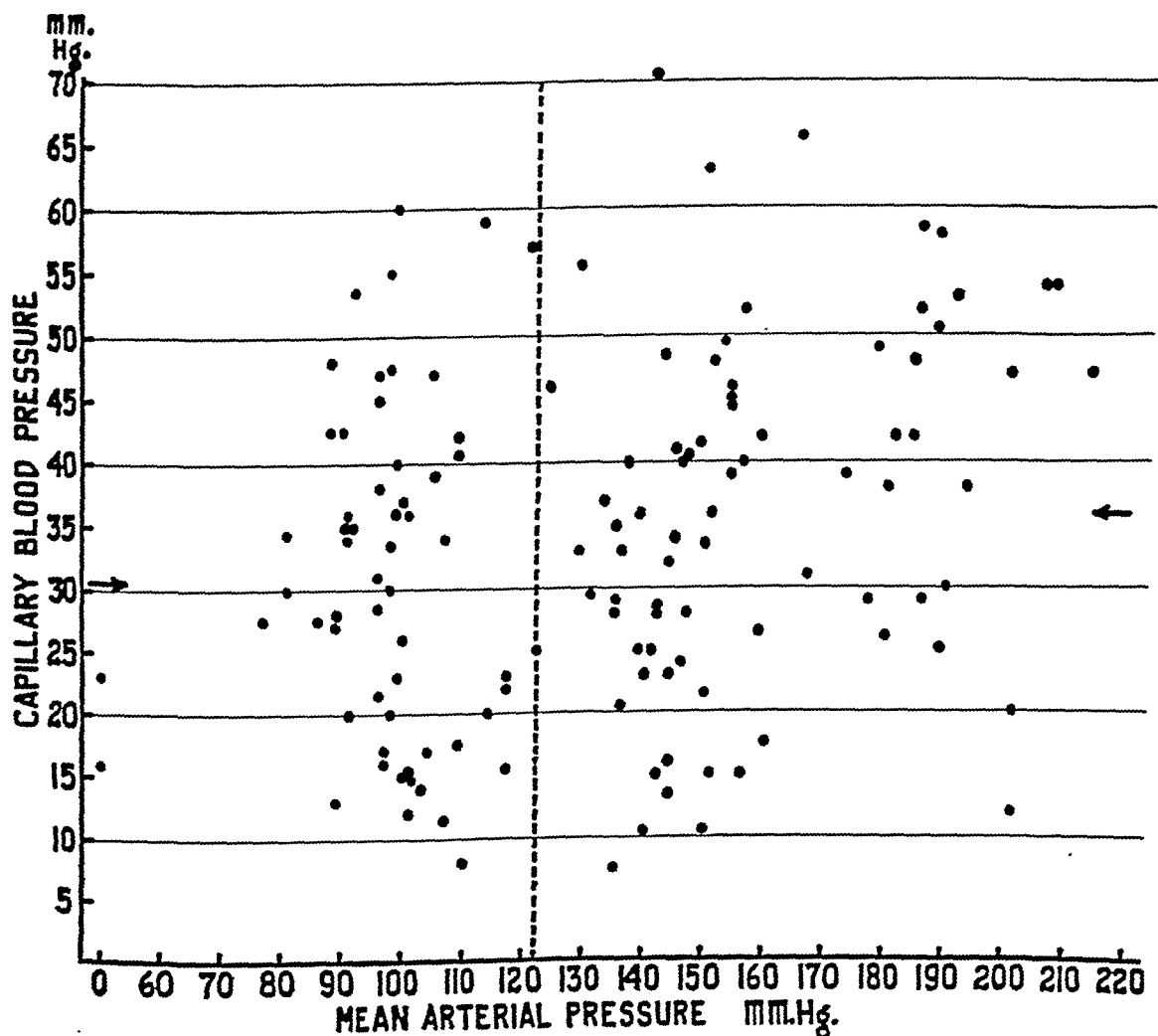


FIG. 8. DIGITAL CAPILLARY BLOOD PRESSURE IN THE ARTERIOLAR LIMBS OF SUBJECTS WITH NORMAL ARTERIAL PRESSURE COMPARED WITH PRESSURES IN THE ARTERIOLAR LIMBS OF HYPERTENSIVE PATIENTS

The capillary blood pressures vary over the same range in both groups of subjects. Due to multiple aneurysms, the brachial arterial pressure was unobtainable in the subject whose mean arterial pressure is recorded as zero. This does not exclude a substantial non-pulsatile arterial pressure.

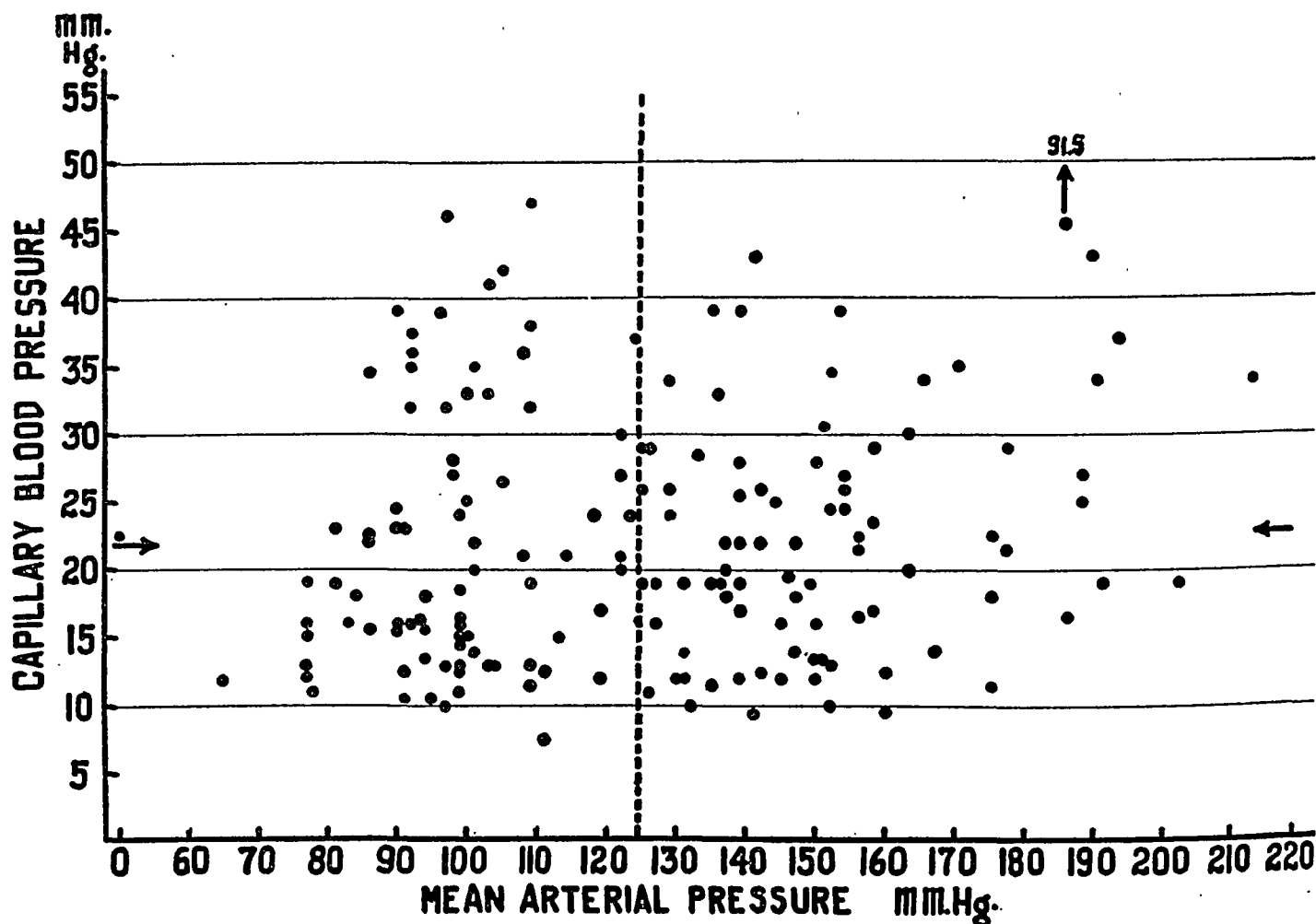


FIG. 9. DIGITAL CAPILLARY BLOOD PRESSURE IN THE VENOUS LIMBS OF SUBJECTS WITH NORMAL ARTERIAL PRESSURE COMPARED WITH PRESSURES IN THE VENOUS LIMBS OF HYPERTENSIVE PATIENTS

The capillary blood pressures vary over the same range in both groups of subjects. The subject whose mean arterial pressure is recorded as zero is the one referred to in Figure 8.

quantitatively similar in the normal and hypertensive subjects, and in the same individual or group, at various levels of blood pressure. This was so not only during the resting state but also during such physiologic influences as neurogenic vasoconstrictions, reactive hyperemia, variations in digital skin temperature between 27°C . and 35°C ., and reflex vasodilatation. Although these influences are known to alter markedly the blood flow to the digits (11, 12), they induced in both normal and hypertensive subjects only comparatively small, and equal, changes (20 per cent to 25 per cent of the initial value) in capillary blood pressure. The resultant values did not fall clearly beyond the limits determined when these influences were not operating. Such changes as did occur were similar in all locations in the capillary, except during reflex vasodilatation, when the pressure in the venous limb of the capillary rose while

that in the arteriolar limb remained unchanged. Only by raising the local venous pressure, was the digital capillary blood pressure consistently altered to values beyond the wide normal limits. Venous pressure was not measured in these subjects. Clinically, there was no evidence that it was increased in any subject. The above changes were similar at all ranges of arterial pressure.

The data do not adequately clarify the mechanism by which these physiologic stimuli induce such great changes in digital blood flow without considerable change in capillary blood pressure. Perhaps the arteriole-venule anastomoses constitute one of the factors in this mechanism. By varying the calibre of the arteriole-venule shunts, and thus the amount of blood which by-passes the capillary, physiologic influences could alter considerably the blood flow through the digit without affecting significantly the digital capillary blood

pressure. Also, the digital blood flow derives from the sum of the flows through a large number of small peripheral vascular units. A relatively small change in a function of each small unit, when multiplied by the large number of units, could account for a considerable change in some function (such as blood flow) of the digit as a whole.

It is significant for the maintenance of the passage of normal amounts of fluid across the capillary membrane that there be a mechanism whereby the capillary blood pressure remains relatively unchanged during the considerable alterations in blood flow which accompany physiologic stimuli. The essentially similar capillary blood pressure in the digits of both normal and hypertensive subjects indicates an increased steepness of the gradient of pressure in the hypertensive subject; and locates it between the artery (elevated pressure) and capillary (normal pressure), presumably in the arteriole. The increased pressure-gradient is apparently due to the increased resistance in the arteriole. This is in harmony with the present concept that the increased general vascular resistance in hypertension is arteriolar in origin. The 50 per cent greater average gradient of pressure from arteriolar limb to ve-

nous limb of the capillary in hypertensive subjects may suggest that the capillaries also contribute to the increased vascular resistance in hypertension. This consideration was discarded because of the wide scattering of values in both groups of subjects and the failure of capillary blood pressure to show any relationship to the arterial pressure. Certainly, the overwhelming portion of the increased vascular resistance in the digits of the hypertensive subjects is arteriolar in origin. These data do not indicate the nature of the increased arteriolar resistance, whether functional (constriction), or the result of some disease process.

If vasodilating procedures relaxed fully the vascular resistance of both hypertensive and normal subjects, then, due to the greater arterial pressure, the capillary blood pressure in the hypertensive subjects should exceed that in normal subjects. The three vasodilating procedures employed, reflex vasodilatation, reactive hyperemia, and histamine injected locally, all produced vasodilatation through relaxation of vascular resistance. These few experiments suggested that only the locally injected histamine appeared to relax, at least to some extent, the increased vas-

TABLE VIII

Capillary blood pressure in the same individual during stages of elevated and lowered arterial pressure

Subject (Sex, age)	Diagnosis	Hypertensive stage						Stage of lowered blood pressure							
		Skin tem- pera- ture	Arterial pressure		Capillary blood pressure			Skin tem- pera- ture	Arterial pressure		Capillary blood pressure				Lowering of arterial pressure due to
			Read- ing	"Mean"	Arte- riolar limb	Sum- mit	Venous limb		Read- ing	"Mean"	Arte- riole	Arte- riolar limb	Sum- mit	Venous limb	
M. E. (F, 30)	Essential hypertension	° C. 29.1	mm. Hg 164/98	mm. Hg 131	mm. Hg	mm. Hg	mm. Hg 14, 12	° C. 33.5	mm. Hg 116/78	mm. Hg 97	mm. Hg 50, 56	mm. Hg	mm. Hg 26.5	mm. Hg	Spontaneous subsidence
E. E. (F, 33)	Essential hypertension	35.3 34.6	192/114 174/126	153 150	49.5 63			33.1 34.8 34.4 35.4 34.2	116/94 126/98 112/90 130/103 135/110	105 124 101 119 123		35 47 30		22 17 24	Bilateral splanchnic and lumbar sympathectomy
J. B. (M, 41)	Subarachnoid hemorrhage	31.4	182/106	144	16, 13.5	20.5		28.6 28.5	148/90 136/85	119 111		23, 32, 19.5	23, 27	12	Spontaneous subsidence
M. W. (M, 26)	Chronic pyelone- phritis	34.2	174/126	150		20, 16	20, 13.5	34.2	130/90	110		8		10, 22, 14	Left nephrectomy
			RIGHT ARM						LEFT ARM						
J. A. (M, 41)	Essential hypertension	31.5 32.0 28.9	148/106 180/110 160/110	127 135 135			16, 19 11.5	32.7 33.2 32.2 33.3	not obtain- able			17.5		7.5 12.5 19, 13 11.5	Local arterial disease reduced left arm pulse- less

cular resistance of the hypertensive subjects, whereas reflex vasodilatation and reactive hyperemia did not. Only during the hyperemia of histamine did the capillary blood pressure in the hypertensive exceed that in the normal subject. During the physiologically induced vasodilatations of reactive hyperemia and reflex vasodilatation, the capillary blood pressure was essentially equal in the two groups. The data do not indicate whether the relaxation of the increased vascular resistance by histamine was complete or partial.

In both normal and hypertensive subjects, these three vasodilating procedures caused a disproportionately great rise in the capillary blood pressure in the venous limb. As a result, the increased digital blood flow of vasodilatation was accompanied by a lower gradient of pressure through the capillaries of the digit. Apparently, digital blood flow need not be proportional to the gradient of digital capillary blood pressure, and may be more closely dependent upon the patency of the arteriole-venule anastomoses. Dilatation of these structures would increase the digital blood flow and, by raising the blood pressure in the venous limb of the capillary, reduce the gradient of pressure through the capillary.

These studies suggest that there are mechanisms which maintain the digital capillary blood pressure within certain limits. These limits appear not to be exceeded during the considerable changes in digital blood flow which result from the action of certain physiologic influences. Nor are these limits exceeded when a disease process, such as essential hypertension, markedly alters the pressure in the arteries.

SUMMARY

1. The digital capillary blood pressure for all locations in the capillary varied within wide limits, and was qualitatively and quantitatively similar in both normal and hypertensive subjects. This maintained during the following observations which apply equally to both groups of subjects.

- a. Such physiologic influences as neurogenic vasoconstrictions, reactive hyperemia, reflex vasodilatation, and variations in skin temperature between 27° C. and 35° C., all induced such comparatively small changes in the digital capillary

blood pressure that the resultant values did not fall beyond "resting" limits. These changes were considerably smaller than the much larger alterations in digital blood flow known to occur under similar circumstances.

- b. Only during increases in local venous pressure did the digital capillary blood pressure consistently exceed "resting" values.

- c. Wide variations in digital capillary blood pressure persisted during reflex vasodilatation, a state during which digital circulation is considered to be full, standard, and reproducible.

- d. During vasodilatation, induced reflexly or by locally injected histamine, there was a disproportionately greater increase in pressure in the venous limb than elsewhere in the capillary. The other states all influenced equally the digital capillary blood pressure in all locations of the capillary.

2. No correlation existed between the digital capillary blood pressure and the arterial pressure, except perhaps during the hyperemia induced by histamine.

3. Some mechanism maintains the digital capillary blood pressure within relatively fixed limits, even during marked changes in digital blood flow and arterial pressure.

4. The similarity of the digital capillary blood pressure of normal and hypertensive subjects indicates that in the digits the increased vascular resistance of hypertensive subjects is precapillary, presumably arteriolar.

5. During the vasodilatation of reactive hyperemia and reflex vasodilatation, the digital capillary blood pressure was essentially equal in both normal and hypertensive subjects; during vasodilatation induced by locally injected histamine, the capillary pressure of the hypertensive subjects exceeded that in normal subjects. These few experiments may suggest that in the digits histamine appeared to relax, at least to some extent, the *increased* vascular resistance of hypertension, whereas reactive hyperemia and reflex vasodilatation did not.

6. These data and the conclusions derived therefrom apply *only* to the capillary blood pressure in the nail-folds of the fingers.

The authors gratefully acknowledge their indebtedness to Mr. William A. Oktavec, Jr., for making the micro-pipettes and for his constant and most helpful assistance in this study.

BIBLIOGRAPHY

1. Weiss, S., and Ellis, L. B., The quantitative aspects and dynamics of the circulatory mechanism in arterial hypertension. *Am. Heart J.*, 1930, 5, 448.
2. Boas, E. P., and Frant, S., Capillary blood pressure in arterial hypertension. *Arch. Int. Med.*, 1922, 30, 40.
3. Boas, E. P., and Mufson, I., Capillary blood pressure in arterial hypertension and nephritis. *J. Lab. and Clin. Med.*, 1923, 9, 152.
4. Ellis, L. B., and Weiss, S., Measurements of capillary pressure under natural conditions and after arteriolar dilatation in normal subjects and in patients with arterial hypertension and with arteriosclerosis. *J. Clin. Invest.*, 1929, 8, 47.
5. Mufson, I., Study of capillary pressure in nephritis and hypertension. *Am. J. M. Sc.*, 1932, 183, 632.
6. Eichna, L. W., and Bordley, J., III, Capillary blood pressure in man. Comparison of direct and indirect methods of measurement. *J. Clin. Invest.*, 1939, 18, 695.
7. Landis, E. M., Microinjection studies of capillary blood pressure in human skin. *Heart*, 1930, 15, 209.
8. Eichna, L. W., and Wilkins, R. W., Capillary blood pressure in man. Direct measurements in the digits during induced vasoconstriction. *J. Clin. Invest.*, 1942, 21, 697.
9. Adson, A. W., Craig, W. M., and Brown, G. E., Surgery in its relation to hypertension. *Surg., Gynec. and Obst.*, 1936, 62 (2A), 314.
10. Grant, R. T., and Bland, E. F., Observations on arteriovenous anastomoses in human skin and in the bird's foot with special reference to the reactions to cold. *Heart*, 1931, 15, 385.
11. Wilkins, R. W., Doupe, J., and Newman, H. W., The rate of blood flow in normal fingers. *Clin. Sc.*, 1938, 3, 403.
12. Burton, A. C., The range and variability of the blood flow in the human fingers and the vaso-motor regulation of body temperature. *Am. J. Physiol.*, 1939, 127, 437.

CAPILLARY BLOOD PRESSURE IN MAN. DIRECT MEASUREMENTS IN THE DIGITS DURING ARTERIAL HYPERTENSION INDUCED BY PAREDROLINOL SULFATE¹

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A previous study (1) indicated that the digital capillary blood pressure was approximately equal in both normal and hypertensive subjects, not only during the resting state, but also during physiologically induced vasoconstrictions and vasodilatations. It was the purpose of this investigation to determine what happened to digital capillary blood pressure when hypertension was induced in subjects with normal arterial pressures.

METHODS

General. As in previous studies (1, 2, 3), all determinations of capillary blood pressure were made on capillaries in the nail-folds of the fingers. The direct microinjection method (Landis) (4), modified as previously described (2), was employed. The general methods, conditions, and precautions employed were fully described in a previous communication (2).

Particular. At both the normal and elevated levels of arterial pressure, capillary blood pressure was always determined in the same location of the same capillary. Both levels of arterial pressure were obtained during a single observation period, throughout which the digital skin temperature was maintained constantly at levels of moderate digital vasodilatation (2). Temporary arterial hypertension of 45 to 60 minutes duration was produced by the intramuscular injection of 20 mgm. to 30 mgm. of paredrolinol sulfate.² This method was chosen because the hypertension thus produced is not accompanied by a significant alteration in cardiac output, velocity of blood flow, basal metabolic rate (5), or skin temperature (6). Moreover, others have suggested that the hypertension induced by paredrolinol sulfate "... presents a condition resembling clinical hypertension ..." (5).

The technical difficulties encountered in determining repeatedly the capillary blood pressure in the same location of a given capillary, limited these observations chiefly to the large capillaries of patients with Raynaud's disease and scleroderma. One normal subject, 2 patients with Raynaud's disease, and one with scleroderma served for this study.

All interruptions of the sympathetic innervation to the fingers were accomplished by operative preganglionic sympathectomy according to the method of Smithwick (7, 8).

RESULTS

Normal sized capillaries—Normal subject

During the temporary hypertension induced in one normal subject, the digital capillary blood pressure in 3 capillaries remained within the limits determined for this subject when his arterial pressure was normal (Figure 1). The hypertension was associated with a decrease of 2 mm. Hg to 7 mm. Hg in the capillary blood pressure in these capillaries. In the arteriolar limb of a fourth capillary, the pressure rose by 19.5 mm. Hg (Figure 1).

Abnormally large capillaries

Sympathetic innervation intact. With the mean arterial pressure elevated to 140 to 150 mm. Hg, the digital capillary blood pressure usually differed but slightly from that determined in the same 3 individuals at their normal mean arterial pressures of 100 to 110 mm. Hg (Figure 1). In 7 summits and venous limbs, the difference amounted to -7.5 mm. Hg to +10.0 mm. Hg, and in one arteriolar limb, to +22 mm. Hg (Figure 1). Only this latter value exceeded the limits established for these subjects when their arterial pressures were normal.

In 4 capillaries of 1 subject, the digital capillary blood pressure was determined during the induced hypertension and then redetermined at the height of the erythema of a histamine-flare, produced at the peak of the hypertension. During the histamine-flare, the digital capillary blood pressure increased by 8 mm. Hg to 17 mm. Hg (Figure 1). The resultant values tended to exceed the upper limits established under resting conditions in the same portions of these capillaries at normal and elevated arterial pressures.

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²The paredrolinol sulfate was kindly supplied by The Smith, Kline and French Laboratories.

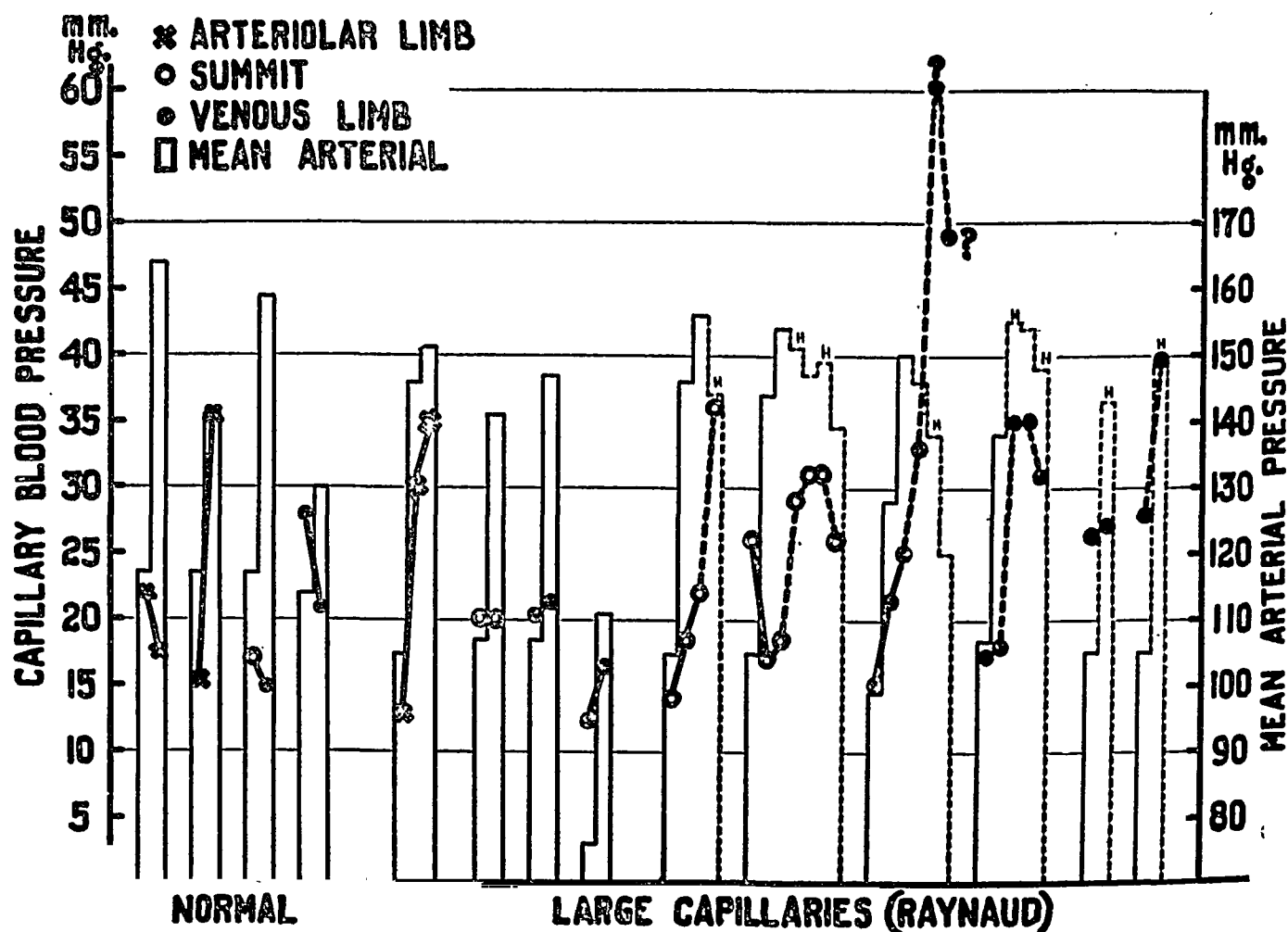


FIG. 1. RELATION BETWEEN MEAN ARTERIAL PRESSURE AND DIGITAL CAPILLARY BLOOD PRESSURE CONCURRENTLY DETERMINED IN THE SAME CAPILLARY AT NORMAL AND INDUCED HYPERTENSIVE LEVELS OF ARTERIAL PRESSURE. DIGITAL INNervation INTACT

First 4 determinations in normal sized capillaries of one normal individual. All other determinations in abnormally large capillaries of 3 subjects with Raynaud's disease and scleroderma. The two values noted with a question mark may have been "arteriole" pressures rather than capillary blood pressures.

Both charts are similarly plotted.

The ordinates indicate in mm. Hg digital capillary blood pressure and mean arterial pressure. Along the abscissae, each unit of the graph represents a *single* capillary. The key in the upper left hand corner gives the symbols representing each type of blood pressure.

The change in mean arterial pressure is indicated by the change in height of the white column. The change in digital capillary blood pressure is indicated by the darker line connecting the various symbols for capillary blood pressure. At all times, concurrent determinations of capillary blood pressure and mean arterial pressure are plotted in the same abscissal space.

Determinations under resting conditions are connected by solid lines. Determinations during local vasodilatation are indicated by the dotted lines. When vasodilatation was induced by histamine, there is a small *H* above the white column, when produced by reactive hyperemia, a small *R* tops the column.

The final values did not exceed the range previously determined in a larger group of subjects with normal arterial pressures (3).

Sympathetic innervation interrupted. Similar results were obtained on the same 2 patients with Raynaud's disease after preganglionic sympathectomy of the upper extremity. When the arterial

pressure increased from normal to hypertensive levels, digital capillary blood pressure in 5 capillaries changed by -2 mm. Hg to $+7$ mm. Hg (Figure 2). Again, the final values remained within the range established for these subjects when their arterial pressures were normal.

During the erythema of histamine-flares, pro-

duced at the peak of the hypertension, digital capillary blood pressure in 3 capillaries rose 4 mm. Hg, 9 mm. Hg, and 13 mm. Hg (Figure 2) above the resting values, obtained at both normal and elevated arterial pressures. When reactive hyperemia was produced at the height of the hypertension, digital capillary blood pressure was less altered, changing by -1 mm. Hg to $+4$ mm. Hg in 3 capillaries and by $+13$ mm. Hg in a fourth (Figure 2). As a result, the final values, obtained during the histamine-flares at hypertensive arterial pressures, tended to exceed the limits of capillary blood pressure for these patients at their normal and hypertensive pressures. During reactive hyperemia at hypertensive arterial pressures, the final capillary blood pressures were not significantly altered from the resting values at normal arterial pressures.

DISCUSSION

Since the experiments are few and the data not entirely uniform, general conclusions are not warranted. However, it did seem of interest that paredrinol sulfate produced in normal individuals a hypertension during which the digital capillary blood pressure remained essentially unchanged. This indicates an increased vascular resistance of precapillary origin, probably arteriolar in location. Since the digital capillary blood pressure during the hypertension induced by paredrinol was normal and approximately equal whether the digits were sympathectomized or whether their innervation was intact, the increased vascular resistance (and hypertension) was not produced by mediation of the sympathetic nervous system, at least not in so far as the fingers are concerned.

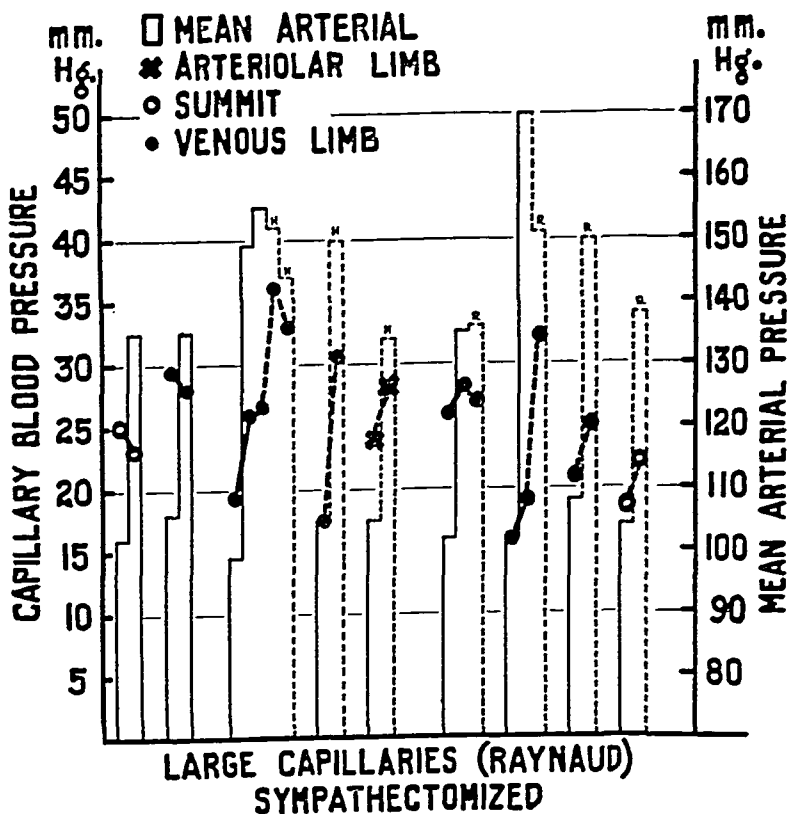


FIG. 2. RELATION BETWEEN MEAN ARTERIAL PRESSURE AND DIGITAL CAPILLARY BLOOD PRESSURE CONCURRENTLY DETERMINED IN THE SAME CAPILLARY AT NORMAL AND INDUCED HYPERTENSIVE LEVELS OF ARTERIAL PRESSURE. PRE-GANGLIONIC SYMPHECTOMY OF DIGITS

All determinations in abnormally large capillaries of 2 subjects with Raynaud's disease.

In several respects, the digital capillary blood pressure during hypertension induced by paredrinol was similar to that in essential hypertension. Each period of hypertension was associated with a normal digital capillary blood pressure; an indication of an increased vascular resistance of precapillary origin. Each hypertensive phase was characterized by a failure of the digital capillary blood pressure to rise significantly during the vasodilatation of reactive hyperemia, though a considerable increase in pressure followed the local injection of histamine. Apparently histamine released, at least to some extent, the increased vascular resistance in the digits, whereas reactive hyperemia failed to do so.

These points of similarity are not sufficient justification for considering the mechanism of essential hypertension to be similar to that induced by paredrinol sulfate. Other types of hypertension may produce similar changes in the digital capillary blood pressure.

SUMMARY

1. During the hypertension induced by paredrinol sulfate in subjects with normal arterial pressures, the digital capillary blood pressure remained within the limits previously established for the same individuals when their arterial pressures were normal.

2. This finding maintained equally for capillaries in digits with intact innervation and after preganglionic sympathectomy.

3. At the height of the paredrinol-induced hypertension, the vasodilatation of local reactive hyperemia did not significantly alter the digital capillary blood pressure. The hyperemia of the histamine-flare was usually associated with a rise

in the digital capillary blood pressure to values which just exceeded the pressures obtained during the resting state at both normal and elevated arterial pressures.

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BIBLIOGRAPHY

1. Eichna, L. W., and Bordley, J., III, Capillary blood pressure in man. Comparison of direct and indirect methods of measurement. *J. Clin. Invest.*, 1939, 18, 695.
2. Eichna, L. W., and Wilkins, R. W., Capillary blood pressure in man. Direct measurements in the digits during induced vasoconstriction. *J. Clin. Invest.*, 1942, 21, 697.
3. Eichna, L. W., and Bordley, J., III, Capillary blood pressure in man. Direct measurements in the digits of normal and hypertensive subjects during vasoconstriction and vasodilatation variously induced. *J. Clin. Invest.*, 1942, 21, 711.
4. Landis, E. M., Microinjection studies of capillary blood pressure in human skin. *Heart*, 1930, 15, 209.
5. Stead, E. A., Jr., and Kunkel, P., Mechanism of the arterial hypertension induced by paredrinol (α -N-dimethyl-p-hydroxyphenethylamine). *J. Clin. Invest.*, 1939, 18, 439.
6. Abbott, W. O., and Henry, C. M., Paredrine (β -4-hydroxyphenylisopropylamine). A clinical investigation of a sympathomimetic drug. *Am. J. M. Sc.*, 1937, 193, 661.
7. Smithwick, R. H., Modified dorsal sympathectomy for vascular spasm (Raynaud's disease) of the upper extremity. A preliminary report. *Ann. Surg.*, 1936, 104, 339.
8. Smithwick, R. H., The value of sympathectomy in the treatment of vascular disease. *New England J. Med.*, 1937, 216, 141.

THE INFLUENCE OF ALTERATIONS IN ACID-BASE BALANCE UPON TRANSFERS OF CARBON DIOXIDE AND BICARBONATE IN MAN¹

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(Received for publication May 15, 1942)

The output of carbon dioxide by the lungs is primarily dependent upon the oxidative metabolism of the organism, and may, indeed, be used as a measure of oxidative processes under appropriate conditions (1 to 3). It is well recognized, however, that augmentation or depletion of the large quantity of CO₂ stored within the body, which occurs during alkalosis or acidosis, may produce alterations in respiratory CO₂ output, independent of oxidative metabolism (4 to 7). Some attempts have been made to establish quantitative relationships between such changes in the output of CO₂ by the lungs and fluctuations of acid-base equilibrium within the organism. Shaw (8) measured the respiratory exchange of cats subjected to artificial ventilation with CO₂-rich mixtures, and correlated the CO₂ exchange of the whole animal with variations in blood CO₂ content. From such observations, the amount of CO₂ absorbed by the tissues could be estimated. Irving and his co-workers (9, 10) made direct determinations of the CO₂ content of various tissues of dogs and cats overventilated with air and with CO₂ enriched mixtures. They attempted to account for the net CO₂ exchange of the whole organism in terms of altered CO₂ content of blood, muscle, bone, and viscera. Applications of Shaw's technique in an effort to determine the CO₂ capacity of the human body has been reported by Adolph, Nance, and Shilling (11). Their results were inconclusive, as were those of similar studies by Brocklehurst and Henderson (12), because it proved impossible to attain the equilibrium state required by the conditions of the experiments (13). In all of these investigations, acid-base change was induced either by overbreathing

or by ventilation with CO₂-rich mixtures, procedures which primarily altered the concentration of dissolved CO₂ in the body (14). No comparable studies have been reported concerning the influence of primary alteration in bicarbonate ion concentration upon respiratory CO₂ production.

The present study deals with the effects of acidosis and alkalosis upon respiratory CO₂ output in the post-absorptive state, when the oxidative metabolic mixture is relatively constant. Concentration of dissolved CO₂ in the body was increased by rebreathing, and decreased by overbreathing; concentration of bicarbonate ion was altered by sodium bicarbonate infusion, and by ammonium chloride ingestion. The respiratory production of CO₂ was found to be strikingly altered during primary change of the concentration of dissolved CO₂ but was little influenced by change of serum bicarbonate.

EXPERIMENTAL PROCEDURES AND METHODS

The experiments involving measurement of respiratory exchange were carried out on normal male adults (the same subject was used in all but 2 experiments). The fasting subject came to the laboratory at about 8 a.m. and rested for one-half hour under basal conditions. The basal respiratory exchange was determined over a 10-minute period by the open circuit method, with the subject breathing through a rubber mouthpiece into a Tissot spirometer. Analyses of the expired air for CO₂ and oxygen were carried out in duplicate by means of a modified Haldane apparatus. The usual precautions were taken to avoid leaks and the apparatus was checked from time to time by analyses of atmospheric air.

Venous blood samples for determination of serum CO₂ content were obtained anaerobically, without stasis, immediately before the initiation of the procedure designed to alter acid-base equilibrium. In the overventilation experiments, as soon as the blood was in the syringe, the subject began to breathe into the spirometer at a ventilation rate about twice normal. After 5 to 10 minutes of overbreathing, a second venepuncture was made and the spirometer disconnected as soon as the blood sample had been obtained. The rebreathing experiments were carried

¹ A preliminary report was presented to the American Society for Clinical Investigation in May 1940. *J. Clin. Invest.*, 1941, 20, 453 (Proc.).

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In several respects, the digital capillary blood pressure during hypertension induced by paredrinol was similar to that in essential hypertension. Each period of hypertension was associated with a normal digital capillary blood pressure; an indication of an increased vascular resistance of precapillary origin. Each hypertensive phase was characterized by a failure of the digital capillary blood pressure to rise significantly during the vasodilatation of reactive hyperemia, though a considerable increase in pressure followed the local injection of histamine. Apparently histamine released, at least to some extent, the increased vascular resistance in the digits, whereas reactive hyperemia failed to do so.

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SUMMARY

1. During the hypertension induced by paredrinol sulfate in subjects with normal arterial pressures, the digital capillary blood pressure remained within the limits previously established for the same individuals when their arterial pressures were normal.

2. This finding maintained equally for capillaries in digits with intact innervation and after preganglionic sympathectomy.

3. At the height of the paredrinol-induced hypertension, the vasodilatation of local reactive hyperemia did not significantly alter the digital capillary blood pressure. The hyperemia of the histamine-flare was usually associated with a rise

CO₂ content in millimols per liter at the beginning of the experiment.

The validity of such calculations of distribution volumes has been discussed by Bourdillon and Lavietes (19).

RESULTS

Overventilation

The carbon dioxide exchange during mild overventilation of 5 to 10 minutes duration was measured in 8 experiments. The results are presented in Table I A and Figure 1. It is apparent that in every experiment the total

quantity of CO₂ given up by the organism exceeded the amount lost from the extracellular fluids alone. A portion must therefore have come from the tissue cells.

The observed fall in serum CO₂ content was very small in the first three experiments; consequently, the cells were credited with a large contribution toward total expired CO₂. It appeared that the peripheral circulatory slowing known to occur during overventilation (20) was masking, in blood drawn from the arm veins, the true fall in serum CO₂ content. Since to

TABLE I
Exchanges of CO₂ during change of acid-base balance

Experiment	Duration	Change of serum CO ₂ content	Oxygen consumption	CO ₂ production	Basal R. Q.	Metabolic CO ₂ output	Non-metabolic CO ₂ balance		
							Total	of ECF	of cells
number	minutes	volumes per cent	cc.	cc.		cc.	cc.	cc.	cc.
A. LOSS OF CO ₂ DURING OVERBREATHING									
1*	6.0	-0.8	1725	2376	0.78	1351	-1025	-150	-875
2*	6.0	-0.2	1800	2298	0.88	1587	-711	-33	-678
3*	5.5	-0.7	1800	2555	0.86	1542	-1013	-117	-996
4	5.6	-2.8	1700	2044	0.80	1364	-677	-466	-211
5	8.5	-2.2	2370	2630	0.81	1932	-698	-367	-331
6	10.5	-3.1	2790	2836	0.78	2181	-655	-518	-137
7	8.2	-3.2	2280	2654	0.81	1850	-804	-534	-270
8	7.0	-3.5	2130	2388	0.73	1562	-826	-515	-311
B. RETENTION OF CO ₂ DURING REBREATHING									
1*	8.0	+1.0	2410	1330	0.78	1871	+541	+155	+386
2*	8.3	-0.5	2750	1200	0.91	2511	+1311	-85	+1396
3*	7.5	-0.4	2153	936	0.87	1867	+931	-75	+1006
4	8.3	+3.4	2749	1361	0.81	2230	+869	+510	+359
5	7.5	+4.4	2210	1050	0.82	1803	+753	+660	+93
6	10.7	+2.0	2920	1700	0.84	2453	+753	+331	+422
7	11.5	+3.4	2960	1480	0.82	2427	+947	+567	+380
C. EXCHANGE OF CO ₂ FOLLOWING INGESTION OF NH ₄ CL									
6b		+0.2	2246	1593	0.73	1642	+49	+33	
5a		-0.4	1933	1542	0.75	1453	-89	-67	
2		-0.5	2200	1790	0.80	1753	-37	-83	
5b		+0.5	1900	1479	0.75	1428	-51	+83	
8a		+0.6	1927	1446	0.77	1489	+43	+100	
7a		+0.7	2234	1578	0.72	1614	+36	+117	
7b		+1.0	1947	1485	0.72	1404	-81	+167	
7c		-1.1	2089	1469	0.72	1506	+37	-184	
6a		-1.1	2149	1560	0.73	1571	+11	-184	
1		+1.3	2300	1845	0.80	1849	+4	+217	
8c		-1.6	1954	1487	0.77	1490	+3	-267	
8b		-1.6	1932	1538	0.77	1510	-28	-267	
6c		+1.7	2160	1726	0.79	1723	-3	+283	
3		+2.5	1923	1498	0.77	1497	-1	+417	
4									

* Peripheral vasodilatation not maintained.

out in similar fashion, except that a Douglas bag without valves, containing 80 to 100 liters of a 4.36 to 5.75 per cent CO_2 -air mixture was substituted for the Tissot spirometer. Samples of air from the Douglas bag were taken for analysis shortly before and immediately after the period of re-breathing.

In the ammonium chloride experiments, measurement of respiratory exchange was carried out over 10-minute periods, under resting conditions, at intervals (usually 45, 60, and 90 minutes) after the ingestion of 10 grams of NH_4Cl in 0.5 gram enteric coated tablets. Blood samples for serum CO_2 determination were taken 10 to 20 minutes before and immediately after each collection of expired air.

Measurement of respiratory exchange during sodium bicarbonate infusion proved impracticable. Untrained subjects could therefore be employed and most of the experiments were carried out on convalescent patients. The volume of distribution of bicarbonate ion was studied following the administration of sodium bicarbonate intravenously as a 4 per cent solution in distilled water. The dose ranged from 10 to 14 grams given over a period of 10 to 45 minutes. In order to insure its quantitative administration, the bicarbonate solution was followed by 200 to 300 cc. of normal saline given through the same infusion set. Blood samples were obtained just before and at one or more intervals, 25 to 120 minutes after the end of the infusion. Complete urine collections were made over the period between each pair of blood samples. Determinations were made of serum CO_2 content, serum chloride concentration, and, in some cases, serum sodium concentration. The urine specimens, preserved and, in some instances, collected under mineral oil, were analyzed for total CO_2 content, chloride concentration, and, in some experiments, sodium concentration. In all experiments, the extracellular fluid volume of the subject was determined by the thiocyanate method (15). Thiocyanate was usually administered on the evening before the bicarbonate infusion, so that change in extracellular fluid during the course of the experiment could be estimated from change in serum SCN concentration, as well as from the alterations in concentration of chloride and sodium in the serum.

All chemical determinations were carried out in duplicate. Serum CO_2 content was determined by the method of Van Slyke and Neill (16), serum chloride by the Hald modification of Patterson's micromethod (16), serum sodium by the method of Hald (17), urine sodium by the method of Butler and Tuthill (16), and urine chloride by the modified Volhard-Harvey titration (16). Thiocyanate was determined colorimetrically with ferric nitrate (18).

CALCULATIONS

The magnitude of change in respiratory CO_2 output produced by altered acid-base equilibrium was calculated as follows:

1. The respiratory quotient of the basal period was calculated from the CO_2 output and oxygen consumption of that period.

2. The oxygen consumed during the period of acid-base change was multiplied by the R. Q. of the basal period to give the oxidative or "metabolic" CO_2 production during

acid-base change. The metabolic CO_2 production was then subtracted from the total CO_2 output during the period of acid-base change to give the non-metabolic CO_2 production. The value for non-metabolic CO_2 thus obtained represented the net increase or decrease of CO_2 in the body as a whole, since urinary excretion during the brief periods of over-ventilation or rebreathing was negligible, and the CO_2 content of the urine following ingestion of ammonium chloride proved to be insignificant.

Change in the amount of CO_2 contained within the extracellular fluids was calculated by multiplying change in the concentration of total CO_2 in the serum by the extracellular fluid volume. In all of the experiments, except those dealing with the effects of NH_4Cl ingestion, blood for estimation of serum CO_2 content was drawn immediately before and after the period over which respiratory exchange was measured. Consequently, the calculated alteration in extracellular fluid content occurred during the same interval over which the CO_2 balance of the whole organism was being measured. The difference between extracellular change and change in the content of the body as a whole was allocated to the tissues.

In the ammonium chloride experiments, the first blood sample was drawn at least 10 minutes before collection of expired air began, in order to avoid possible overventilation during the measurement of respiratory exchange. The change in serum CO_2 content over the 10 minute respiratory exchange period was interpolated from the alteration observed over the longer interval, which was usually 20 minutes and never exceeded 30 minutes.

The volume of distribution of administered bicarbonate ion was calculated by means of the following formulae:

$$ECF_2 = \frac{ECF_1 \times [\text{Cl}]_1 + \Delta\text{Cl}}{[\text{Cl}]_2}, \quad (1)$$

where ECF_2 is the volume in liters of extracellular fluid at the end of the experimental period,

ECF_1 is the volume in liters of extracellular fluid at the beginning of the experimental period,

$[\text{Cl}]_1$ is the concentration of serum chloride in milliequivalents per liter at the beginning of the experiment,

ΔCl is the amount of chloride in milliequivalents retained during the experiment,

and $[\text{Cl}]_2$ is the concentration of serum chloride in milliequivalents at the end of the experiment.

ECF_2 was also calculated from change in serum sodium and change in serum SCN by analogous formulae, substituting $[\text{Na}]$ or $[\text{SCN}]$ for $[\text{Cl}]$.

$$\Delta ECF = ECF_2 - ECF_1, \quad (2)$$

where ΔECF is the change in liters in the volume of extracellular fluid during the experiment.

$$V_{\text{HCO}_3} = \frac{\Delta\text{CO}_2 - \Delta ECF[\text{CO}_2]_2}{[\text{CO}_2]_2 - [\text{CO}_2]_1}, \quad (3)$$

where V_{HCO_3} is the volume of distribution of administered bicarbonate, ΔCO_2 is the CO_2 balance in millimols (total CO_2 given as bicarbonate less total CO_2 excreted in the urine), $[\text{CO}_2]_2$ is the serum CO_2 content in millimols per liter at the end of the experiment and $[\text{CO}_2]_1$ is the serum

CO₂ content in millimols per liter at the beginning of the experiment.

The validity of such calculations of distribution volumes has been discussed by Bourdillon and Laviétes (19).

RESULTS

Overventilation

The carbon dioxide exchange during mild over-ventilation of 5 to 10 minutes duration was measured in 8 experiments. The results are presented in Table I A and Figure 1. It is apparent that in every experiment the total

quantity of CO₂ given up by the organism exceeded the amount lost from the extracellular fluids alone. A portion must therefore have come from the tissue cells.

The observed fall in serum CO₂ content was very small in the first three experiments; consequently, the cells were credited with a large contribution toward total expired CO₂. It appeared that the peripheral circulatory slowing known to occur during overventilation (20) was masking, in blood drawn from the arm veins, the true fall in serum CO₂ content. Since to

TABLE I
Exchanges of CO₂ during change of acid-base balance

Experiment	Duration	Change of serum CO ₂ content	Oxygen consumption	CO ₂ production	Basal R. Q.	Metabolic CO ₂ output	Non-metabolic CO ₂ balance		
							Total	of ECF	of cells
number	minutes	volumes per cent	cc.	cc.		cc.	cc.	cc.	
A. LOSS OF CO ₂ DURING OVERBREATHING									
1*	6.0	-0.8	1725	2376	0.78	1351	-1025	-150	-875
2*	6.0	-0.2	1800	2298	0.88	1587	-711	-33	-678
3*	5.5	-0.7	1800	2555	0.86	1542	-1013	-117	-996
4	5.6	-2.8	1700	2044	0.80	1364	-677	-466	-211
5	8.5	-2.2	2370	2630	0.81	1932	-698	-367	-331
6	10.5	-3.1	2790	2836	0.78	2181	-655	-518	-137
7	8.2	-3.2	2280	2654	0.81	1850	-804	-534	-270
8	7.0	-3.5	2130	2388	0.73	1562	-826	-515	-311
B. RETENTION OF CO ₂ DURING REBREATHING									
1*	8.0	+1.0	2410	1330	0.78	1871	+541	+155	+386
2*	8.3	-0.5	2750	1200	0.91	2511	+1311	-85	+1396
3*	7.5	-0.4	2153	936	0.87	1867	+931	-75	+1006
4	8.3	+3.4	2749	1361	0.81	2230	+869	+510	+359
5	7.5	+4.4	2210	1050	0.82	1803	+753	+660	+93
6	10.7	+2.0	2920	1700	0.84	2453	+753	+331	+422
7	11.5	+3.4	2960	1480	0.82	2427	+947	+567	+380
C. EXCHANGE OF CO ₂ FOLLOWING INGESTION OF NH ₄ CL									
6b		+0.2	2246	1593	0.73	1642	+49	+33	
5a		-0.4	1933	1542	0.75	1453	-89	-67	
2		-0.5	2200	1790	0.80	1753	-37	-83	
5b		+0.5	1900	1479	0.75	1428	-51	+83	
8a		+0.6	1927	1446	0.77	1489	+43	+100	
7a		+0.7	2234	1578	0.72	1614	+36	+117	
7b		+1.0	1947	1485	0.72	1404	-81	+167	
7c		-1.1	2089	1469	0.72	1506	+37	-184	
6a		-1.1	2149	1560	0.73	1571	+11	-184	
1		-1.1	2300	1845	0.80	1849	+4	-184	
8c		+1.3	1929	1487	0.77	1490	+3	+217	
8b		-1.6	1954	1538	0.77	1510	-28	-267	
6c		-1.6	1932	1444	0.73	1412	-32	-267	
3		+1.7	2160	1726	0.79	1723	-3	+283	
4		+2.5	1923	1498	0.77	1497	-1	+417	

* Peripheral vasodilatation not maintained.

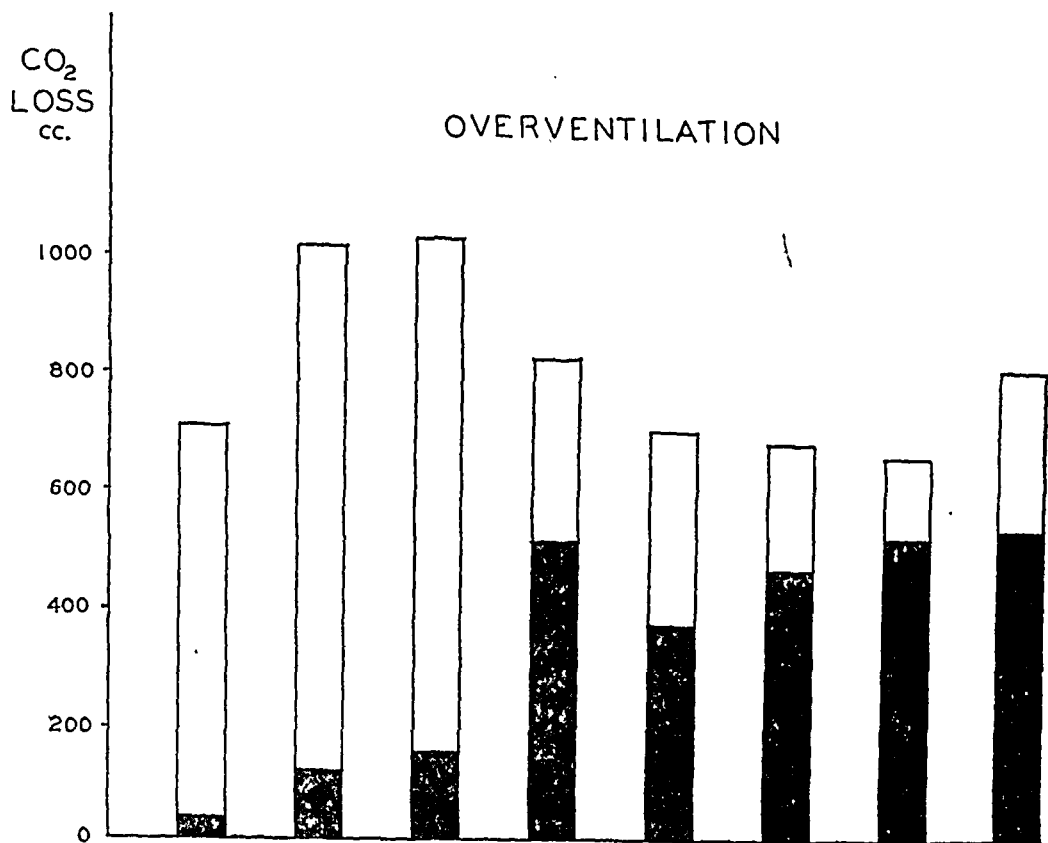


FIG. 1. LOSS OF CO₂ DURING OVERVENTILATION

The total height of each column represents the total loss of nonmetabolic CO₂. The solid portion represents the amount lost from the extracellular fluids. The unshaded portion indicates the amount lost from the cells.

obtain mixed venous blood was hardly feasible, the samples were rendered approximately arterial in subsequent experiments by immersing the arm in hot water (21). Under these conditions, the observed change in serum CO₂ content increased and a more reasonable estimate of intra- and extracellular losses of CO₂ was obtained (Experiments 4 to 8, Table I A).

Rebreathing

Seven experiments were carried out in order to study the effects of rebreathing a CO₂-air mixture for 5 to 10 minutes. The data are presented in Table I B and Figure 2. In none of the experiments did the extracellular fluids accommodate all of the CO₂ retained by the body; hence, the tissue cells must have participated in the storage. In the first 3 experiments, local circulatory effects were even more apparent than in the observations on overventilation. Respiratory CO₂ excess produces local vasodilatation (20) which may completely mask, in peripheral venous blood, the rise of CO₂ content associated

with rebreathing. Blood drawn after rebreathing was uniformly brighter red than that obtained before rebreathing, although no mechanical stasis was utilized during either venepuncture. When peripheral circulatory changes were minimized by immersion of the arm in hot water throughout the experiment, a consistently greater rise in venous CO₂ concentration was observed and a more reliable estimate of extracellular retention of CO₂ was obtained (Experiments 4 to 7, Table I B).

Ammonium chloride ingestion

The results of 15 determinations of carbon dioxide exchange following the ingestion of ammonium chloride are presented in Table I C and Figure 3. There was considerable fluctuation in serum CO₂ content in the first 2 hours after ingestion of NH₄Cl, with a general tendency for a fall to occur during this period. The sporadic increases of serum CO₂ content were attributed to erratic absorption of the enteric coated salt, and to probable stimulation of gastric secretion

(suggested by the occurrence of slight nausea in some experiments). No alterations in respiratory rate were observed during the experiments, nor was there significant variation in the volume of expired air collected in the spirometer over 10-minute periods. Alterations in serum CO_2 content could therefore be attributed with confidence to primary alteration in bicarbonate ion concentration.

In all but 2 experimental periods, in which serum CO_2 was practically constant, the alteration in serum CO_2 content indicated that the extracellular fluids had gained or lost quantities of carbon dioxide, much in excess of any change in the CO_2 content of the whole organism as measured by the respiratory output. Indeed, some of the observations indicated that a considerable loss of CO_2 from the extracellular fluid could occur while the respiratory output actually declined. There was, therefore, not even a directional correlation between the carbon diox-

ide balance of the extracellular fluids and of the subject as a whole under the conditions of these observations. None of the discrepancies in CO_2 exchange could be explained by urinary excretion, since this was negligible. With a very few exceptions, the fluctuations in respiratory output of carbon dioxide were extremely small, exceeding 3 per cent of the total output for 10 minutes in only 3 instances, and never exceeding 6 per cent. If the change in extracellular CO_2 content had been reflected in the respiratory production, the observed changes would have been greater than 3 per cent in all but 1 period, and would have ranged from 6 to 28 per cent in all but 4 periods.

Bicarbonate distribution

Data pertaining to the volume of distribution of bicarbonate ion, administered intravenously as sodium bicarbonate in 7 experiments, are presented in Table II and Figure 4.

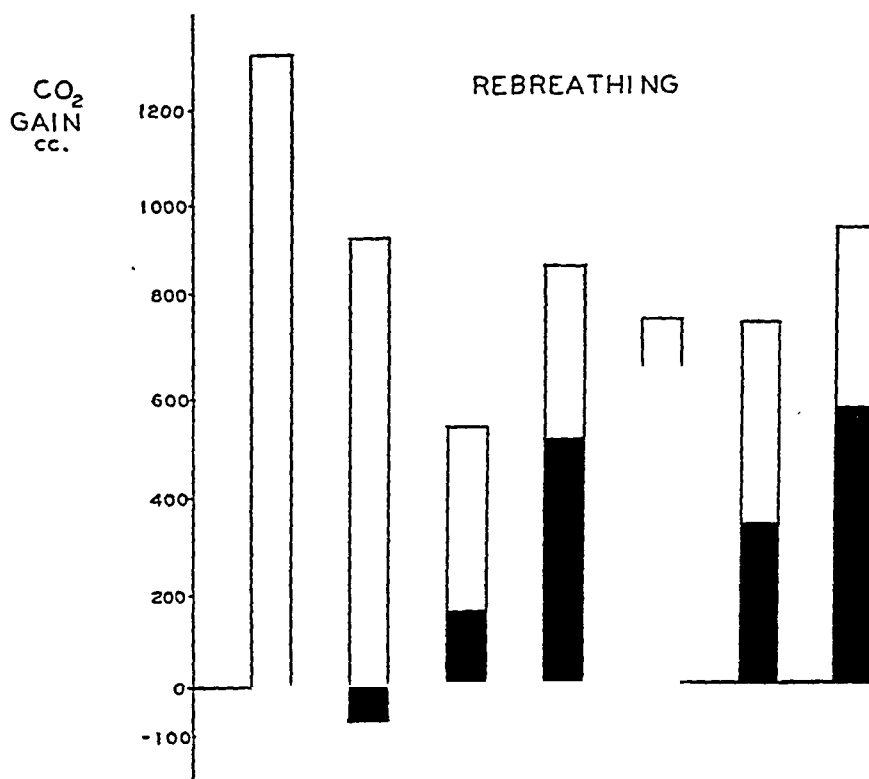


FIG. 2. RETENTION OF CO_2 DURING REBREATHING

Total retention of CO_2 is represented by the total height of each column. The solid portion represents extracellular retention, the unshaded portion, intracellular retention.

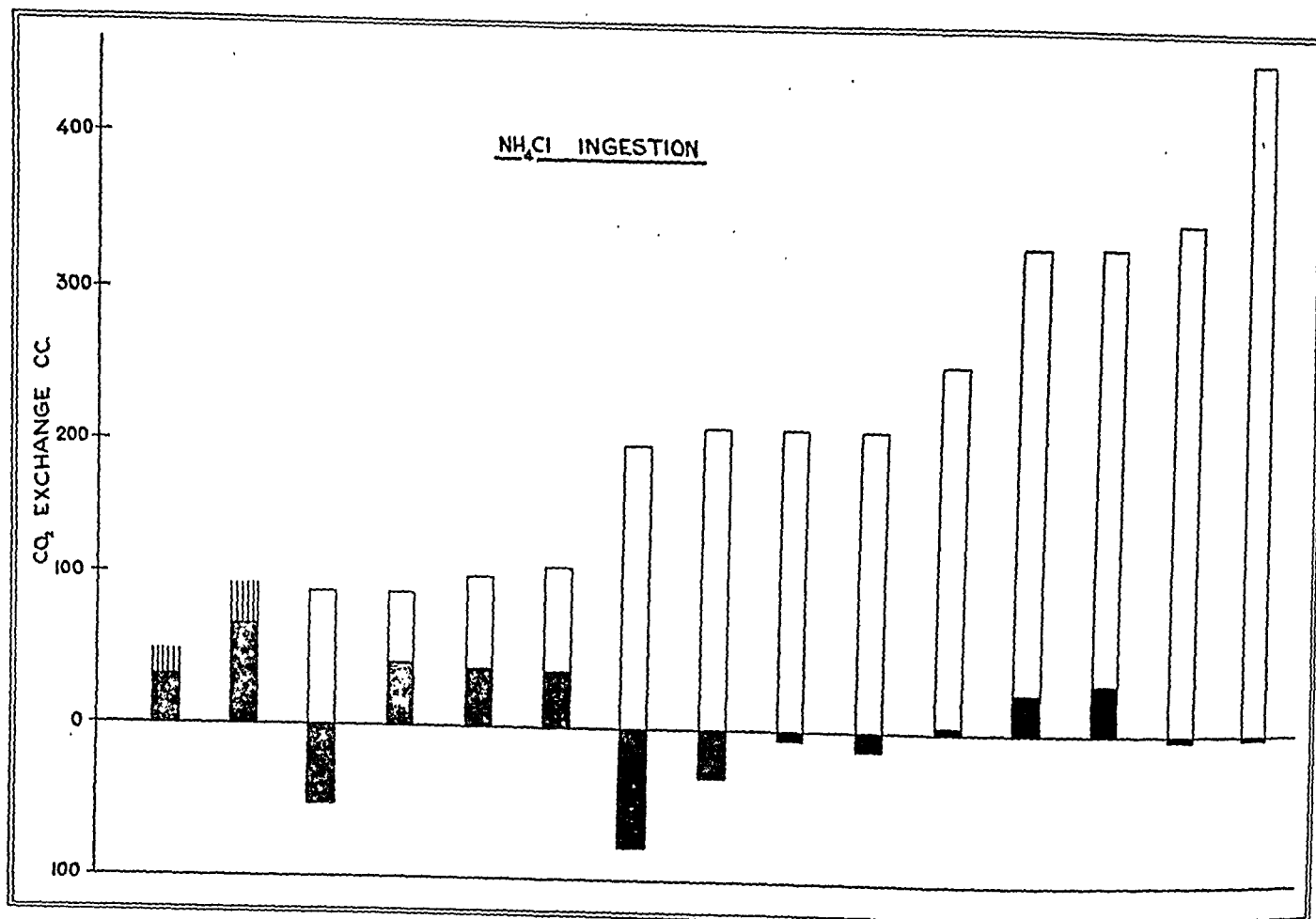


FIG. 3. EXCHANGES OF CO₂ FOLLOWING INGESTION OF NH₄Cl

The CO₂ balance of the extracellular fluids (whether positive or negative) is represented by the total height of each column. The corresponding CO₂ balance of the body as a whole is indicated by the solid portion of the column. The vertical lines above the first 2 columns indicate change in total CO₂ in excess of the extracellular change.

In the majority of experiments, the volume through which the retained bicarbonate ion was distributed approximated the extracellular fluid volume of the subject as measured by the thiocyanate method. In the first experiment, bicarbonate space was apparently less than thiocyanate space. However, in this instance, no estimation of urinary chloride excretion was made. It is clear from the formulae given for calculation of distribution volume that neglecting chloride excretion will, by increasing the value given ΔCl in formula (1), increase the value of ECF_2 . This in turn will increase the value of ΔECF (Formula (2)). Too high a value for ΔECF will give too low a value for V_{HCO_3} in Formula (3). Obviously, therefore, the figures given for bicarbonate space in Experiments 1a and 1b are too low. Furthermore, the value obtained for the same subject in the next experiment was considerably higher and

agreed well with the distribution volume of thiocyanate. The volume calculated for bicarbonate distribution in Experiment 4 may also be in error since, because of considerable difficulty with venepuncture, some stasis was employed. Interpretation is further complicated because the patient also received sodium sulfadiazine intravenously in connection with another study. In 4 of the remaining 5 experiments, agreement between bicarbonate space and thiocyanate space was very close. The discrepancy in Experiment 3, carried out on a 37-year-old woman suffering from an agitated depression, is unexplained.

The possible summation of errors involved in the calculation of bicarbonate distribution volume may amount to several liters. For this, there are two chief sources. The first lies in the estimation of change in extracellular fluid volume, which depends upon small alterations in serum chloride, sodium, or SCN concentrations,

TABLE II
The distribution of intravenous HCO_3^-

Experiment	Subject	Duration	HCO_3^- retained	Change of serum concentration of				Change of ECF volume in liters calculated from ¹			Volume of distribution of added HCO_3^- in liters calculated from ²			Final extra-cellular fluid volume
				CO_2	SCN	Cl	Na	SCN	Cl	Na	SCN	Cl	Na	
number		minutes	m. eq.	m. eq. per liter	mgm. per cent	m. eq. per liter	m. eq. per liter							liters
1a	JDR	25	109.0	+7.7		-5.0			+0.8			10.6		17.5
1b	JDR	75	87.0	+5.0		-5.1			+0.8			12.3		17.5
2	JDR	68	83.4	+4.4		-1.8			+0.4			16.0		17.1
3a	Ba	40	121.1	+4.9		-1.0			-0.05			25.0		15.9
3b	Ba	120	95.4	+3.9		-2.0			-0.08			25.1		15.8
4	Was	30	148.3	+6.6	-0.45	-1.6		+1.2	+0.5		15.9	19.7		21.0
5	Wat	90	117.8	+3.5	-0.57	-3.0		+0.8	+0.4		26.1	30.0		19.1
6	We	40	121.9	+4.1	-0.47	-2.4	+2.0	+0.9	+0.4	+0.7	22.6	26.6	24.1	19.7
7	Me	60	132.1	+5.7	-0.60	-2.5	+3.2	+0.8	+0.6	+0.8	18.2	19.4	18.2	17.2

¹ By formula (1) of text.

² By formula (2) of text.

during the experimental period. Change in serum chloride concentration rarely exceeded 3 milliequivalents per liter. Since the possible error of each determination is approximately 1 milliequivalent, the error of the difference between 2 estimations may reach 2 milliequivalents per liter. Reference to formula (1) shows that the error in estimating ECF change may consequently reach 0.4 liter. This error in ΔECF would produce an error of 2 liters in $V_{\text{HCO}_3^-}$.

(Formula (3)). The importance of this source of error is apparent in the last 4 experiments, where significant differences may be noted among the changes in extracellular fluid volume calculated from thiocyanate, chloride, and sodium concentrations, respectively. When all three ions were determined, the best agreement was obtained between thiocyanate space and sodium space.

The second significant source of error lies in

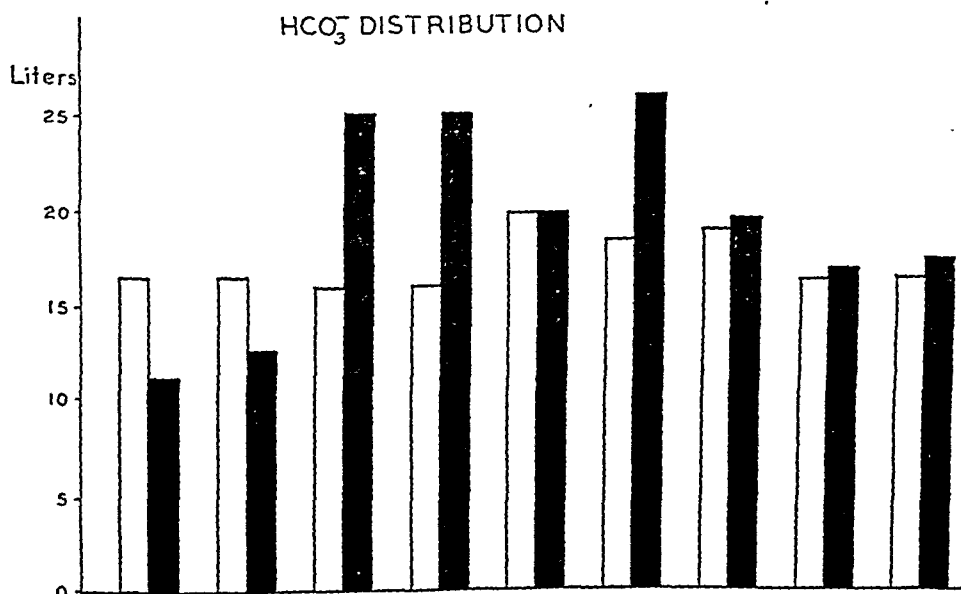


FIG. 4. APPARENT VOLUME OF DISTRIBUTION OF INTRAVENOUS BICARBONATE

The volumes of distribution of bicarbonate ion are represented by the solid columns. The extracellular fluid volumes of the subjects are indicated by the open columns.

estimating change in serum CO_2 content. The error of the chemical determination is not more than 0.15 m.eq. per liter, so that the maximum error of the difference between 2 determinations is 0.3 m.eq. Reference to Formula (3) indicates that this possible error of 0.3 m.eq. will produce an error of about one liter in the calculated bicarbonate distribution volume. Moreover, slight changes in peripheral blood flow can lead to change in venous serum CO_2 content, unrelated to bicarbonate administration.

Since the respiratory output of CO_2 could not be measured over the relatively long periods required for determination of the distribution volume of administered bicarbonate, the possibility of alteration in the total CO_2 content of the organism due to changes in ventilation cannot be excluded. The occurrence of such changes would lead to an error in the estimation of the CO_2 balance (ΔCO_2 , formula (3)), which was taken as the difference between CO_2 administered intravenously as bicarbonate, and total CO_2 excreted in the urine. Retention of CO_2 , due to decreased ventilation, would therefore lead to an erroneously low value for the distribution volume of bicarbonate. However, previous studies have shown that sodium bicarbonate infusions either increase ventilation rate (22 to 25) or have no effect on the breathing (23, 26). Consequently, failure to measure respiratory production of carbon dioxide could lead only to erroneously high values for the distribution volume of administered bicarbonate ion, but could not cause low results.

DISCUSSION

Rebreathing and overbreathing, even when of brief duration, change the carbon dioxide content of the body considerably. A significant portion of the total change in CO_2 content is accounted for by altered concentration within the tissue cells. Since the primary effect of overbreathing or rebreathing is to lower or raise, respectively, the CO_2 tension of the blood (14), the presumption is strong that carbon dioxide can enter or leave the tissue cells in the form of dissolved CO_2 . Direct demonstration of cellular permeability to dissolved CO_2 has been accomplished by Lowry and Hastings (27), using isolated rat

muscle. The existence of similar permeability in the intact animal is also suggested by the observations of Irving and coworkers (9, 10), and of Shaw (8, 13, 28).

Free permeability of cell membranes to dissolved CO_2 implies that, under equilibrium conditions, CO_2 tension in the extracellular fluids and in the cell water shall be equal. If CO_2 tension is lowered in the blood and extracellular fluids by overbreathing, the tissues should give up enough CO_2 to lower the tension in cell water to the same degree. Under these circumstances the amount of CO_2 lost from the *ECF* and cell water will be proportional to the respective volumes of these fluid compartments, except insofar as their CO_2 absorption curves may differ. Irving, Foster and Ferguson (29) have shown that the CO_2 absorption curve of cat muscle is not dissimilar from that of the blood. It seems justifiable, therefore, to assume that under equilibrium conditions the contribution of the tissues in exchanges of dissolved CO_2 should be approximately twice that of the extracellular fluids, since their volumes are in a ratio of about 2 : 1 (30). Failure to observe such proportionality in either the rebreathing or overbreathing experiments suggests that equilibrium was not attained. But a lag in exchange of CO_2 between cells and extracellular fluid may occur because of the absence of carbonic anhydrase from tissue cells (31). The exchanges between alveoli and blood and between blood and extracellular fluid on the other hand are enormously accelerated by the carbonic anhydrase present in red cells. Failure to attain equilibrium was therefore to be expected and the relative amounts of CO_2 lost from or gained by extracellular fluids and intracellular water are of little importance.

The studies of bicarbonate distribution indicate quite clearly that the tissue cells are impermeable to CO_2 bound as bicarbonate. This observation is in keeping with the demonstration by Lowry and Hastings (27) that the cells of isolated rat muscle, although permeable to dissolved CO_2 , are impermeable to bicarbonate ion. Previous studies on human subjects by Palmer and Van Slyke (32) and by Hartmann and Senn (33) led to the conclusion that ingested or intravenously administered sodium bicarbonate was distributed through the total volume of body

water. However, in neither investigation was account taken of urinary excretion of bicarbonate, nor was correction for expansion of extracellular fluid volume attempted. Neglect of either of these factors would give an erroneously high value for the calculated volume of distribution of the administered bicarbonate. Furthermore, many of the observations were on patients with acidosis and dehydration, whose serum bicarbonate concentrations and extracellular fluid volumes were subject to considerable change independent of bicarbonate administration.

Shaw and Messer (28) have reported the changes of serum CO_2 content in 5 cats following intravenous administration of sodium bicarbonate. Since ureteral ligation was carried out prior to injection of the hypertonic bicarbonate solution, the volume through which the bicarbonate was distributed can be calculated from their data, with the assumptions that no urinary excretion occurred, and that enough water left the tissues to restore osmotic equilibrium between cell water and extracellular fluid. If the volume of extracellular fluid in the cat is estimated to be 30 per cent of the body weight, the calculated values for the volume of distribution of bicarbonate ion range from 31.5 to 42.3 per cent of the body weight. Although these values must be considered approximations, they indicate that the bicarbonate space of the cat is far less than the total volume of body water, but agrees fairly well with the volume of extracellular fluids.

The possibility that cell membranes might exhibit a differential permeability to CO_2 and bicarbonate ion was considered in 1920 when Jacobs (34, 35) reported a group of ingenious experiments on plants, protozoa, and amphibia, which suggested strongly that the cell membranes studied were penetrated much more rapidly by carbon dioxide than by bicarbonate ion. Jacobs also inferred from observations on taste sensation in man that carbon dioxide could enter mammalian cells which were relatively impermeable to bicarbonate ions. Subsequently, Gesell (23, 36) supported Jacobs' views concerning cellular impermeability to bicarbonate ion, but his observations were largely concerned with the permeability of the respiratory center, and the evidence obtained was quite indirect or chiefly

inferential. Gesell (37), and other advocates (38, 39) of the theory that the activity of the respiratory center is chiefly determined by local hydrogen ion concentration, have argued on the basis of Jacobs' observations that the apparent specificity of CO_2 as a respiratory stimulant is merely a manifestation of its rapid effect upon the intracellular pH of the center. The observations reported here support Jacobs' hypothesis concerning cellular impermeability to bicarbonate ion. However, recent investigations of respiratory function (40, 41) indicate that the respiratory responses to CO_2 are not merely a manifestation of intracellular pH change.

The effects of ammonium chloride ingestion differed strikingly from those of either rebreathing or overbreathing. Respiratory CO_2 output was not materially altered despite significant losses or gains of CO_2 by the blood and extracellular fluids. Since urinary excretion was negligible, CO_2 leaving the extracellular fluids must have entered the tissue cells. The apparent paradox of an increase in cellular total CO_2 content during fall in CO_2 content of the extracellular fluids is resolved when concentrations of bicarbonate ion and dissolved CO_2 are considered independently. Ammonium chloride acts as would the addition of hydrochloric acid to the blood. That is, it decreases bicarbonate ion concentration, but increases CO_2 tension (14). Depression of bicarbonate concentration in serum does not cause bicarbonate to emerge from the tissues, because of the impermeability of the cell membranes. The increased CO_2 tension, however, results in transfer of dissolved CO_2 from extracellular fluids to the tissues. Consequently, the total CO_2 content of the blood falls, that of the tissues rises, and, by a sort of internal compensation, little or no CO_2 is left for excretion by the lungs. During periods when bicarbonate ion concentration increases, the reverse sequence of events probably occurs. With further depression of serum bicarbonate, and consequently greater increment in CO_2 tension, CO_2 excretion by the lungs would presumably increase, due to stimulation of the respiratory center. Acidosis cannot increase indefinitely without leading to overventilation. It is noteworthy, however, that many attempts to induce overbreathing by ingestion of acid salts (26, 40, 42), and even by

infusion of mineral and organic acids (26, 42 to 44), have produced most undramatic results.

The influence of acidosis upon respiratory output of CO_2 appears therefore to depend upon the type of acid-base change involved. It is well recognized that during the development of or recovery from diabetic acidosis considerable depletion of serum bicarbonate may be unassociated with overventilation (45, 46, 47). Ketone acids may well act like ingested ammonium chloride. They enter the blood stream from the liver (48) and liberate CO_2 from the bicarbonate of the extracellular fluid. But intracellular stores of CO_2 are preserved because bicarbonate cannot leave the cells and ketone acids that enter convert little intracellular bicarbonate to CO_2 , because they are so rapidly oxidized (49). Only when acidosis progresses and overventilation supervenes, will the bicarbonate of cells be depleted. An entirely different situation exists when lactic acid is produced intracellularly. Cell bicarbonate is converted to free CO_2 , which diffuses out into the extracellular fluids. Moreover, the lactic acid itself diffuses out of the cells to liberate more CO_2 extracellularly and the gas can only escape through the lungs. Because of the preponderant contribution by the tissues, large increments in the respiratory production of CO_2 may be observed, with only relatively small changes in the CO_2 content of serum (50).

By virtue of their different distribution and diffusibility, the determinants of the acid-base system achieve a considerable degree of independence. Changes in the CO_2 content of the blood can be correlated with respiratory carbon-dioxide production and with the acid-base balance of the organism as a whole only if the individual variation of each dimension of the system is taken into account.

Because of the uncertain significance of respiratory quotients determined during alteration in acid-base equilibrium, the possibility of calculating metabolic CO_2 production from total CO_2 output, by correcting for the effects of acid-base change, should be considered. Since alterations of CO_2 tension are manifested throughout the body fluid, they will usually have a greater influence upon the respiratory output of CO_2 than will variations in bicarbonate which are

limited to the extracellular compartment. Non-metabolic CO_2 production due to change of bicarbonate ion may be readily calculated as the product of change in serum bicarbonate concentration by the extracellular fluid volume. But estimation of non-metabolic CO_2 related to change in CO_2 tension is subject to several important limitations. Determination of the CO_2 tension of the blood, either by analysis of alveolar air or by calculation from concentrations of bicarbonate and hydrogen ions in serum, involves a minimum possible error of 1.0 mm. of mercury. It can be shown that this error alone would make calculation of metabolic CO_2 production over short periods quite unreliable. Moreover, this calculation requires a knowledge of the CO_2 absorption curve of the tissues, which has not been determined for man, and can only be approximated from the absorption curve for cat muscle determined by Irving, Foster and Ferguson (29). Finally, since the CO_2 tension of the tissues may well fail to attain equilibrium with that of the blood during rapid fluctuations in acid-base equilibrium, estimation of alterations in the carbon dioxide content of the tissues is rendered even more hazardous. Consequently, quantitative correction of overall respiratory CO_2 output for non-metabolic CO_2 does not seem feasible at present.

SUMMARY AND CONCLUSIONS

The influence of changes in acid-base equilibrium upon the output of carbon dioxide by the lungs was studied in human subjects.

Oventilation produced large increments in respiratory output of CO_2 . A portion of the CO_2 was given up by the tissues.

Ventilation with a CO_2 -enriched air caused a marked diminution in the volume of CO_2 given out by the lungs. Part of the CO_2 retention was intracellular.

Alterations of the CO_2 content of blood, produced by ingestion of ammonium chloride, may be unassociated with any significant change in the output of carbon dioxide by the lungs.

The volume of distribution of bicarbonate ion administered intravenously as sodium bicarbonate was found to approximate the extracellular fluid volume as determined by the thiocyanate method.

The observations indicate that the tissue cells of man are freely permeable to dissolved molecular CO_2 , but are impermeable to bicarbonate ion.

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BIBLIOGRAPHY

- Adams, T. W., and Poulton, E. P., A new study of heat production in man. Part III. Guy's Hosp. Reports, 1935, 85, 447 (Series IV, vol. 15).
- King, J. T., Jr., Determination of the basal metabolism from the carbon-dioxide elimination. Bull. Johns Hopkins Hosp., 1921, 32, 277.
- King, J. T., Jr., Basal Metabolism. Williams and Wilkins, Baltimore, 1924.
- Carpenter, T. M., and Lee, R. C., The parallel determination of the R. Q. and alveolar air of man in the post-absorptive condition. J. Nutrition, 1933, 6, 37.
- Lusk, G., The Science of Nutrition. Fourth Edition, reset. W. B. Saunders, Philadelphia, 1928, pp. 96-97.
- Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry. Volume I. Interpretations. Williams and Wilkins, Baltimore, 1931, pp. 15-18.
- Richardson, H. B., The respiratory quotient. Physiol. Rev., 1929, 9, 61.
- Shaw, L. A., The comparative capacity of the blood and of the tissue to absorb carbonic acid. Am. J. Physiol., 1926-27, 79, 91.
- Irving, L., Ferguson, J. K. W., and Plewes, F. B., The source of CO_2 expired and the site of its retention. J. Physiol., 1930, 69, 113.
- Irving, L., and Foster, H. C., The respiratory quotient of resting mammalian muscle as shown by the eviscerated decapitated cat. Am. J. Physiol., 1930, 95, 429.
- Adolph, E. F., Nance, F. D., and Shilling, M. S., The CO_2 capacity of the human body and the progressive effects of CO_2 upon the breathing. Am. J. Physiol., 1928, 87, 532.
- Brocklehurst, R. J., and Henderson, Y., The buffering capacity of the tissues as indicated by the CO_2 capacity of the body. J. Biol. Chem., 1927, 72, 665.
- Shaw, L. A., and Messer, A. C., The carbon dioxide capacity of the body and the rate at which the body comes into equilibrium with changes in alveolar carbon dioxide tension. Am. J. Physiol., 1930, 93, 422.
- Shock, N. W., and Hastings, A. B., Studies on the acid-base balance of the blood. IV. Characterization and interpretation of the acid-base balance. J. Biol. Chem., 1935, 112, 239.
- Lavietes, P. H., Bourdillon, J., and Klinghoffer, K. A., The volume of the extracellular fluids of the body. J. Clin. Invest., 1936, 15, 261.
- Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry. Volume II. Methods. Williams and Wilkins, Baltimore, 1932.
- Hald, P. M., The determination of the bases of serum and whole blood. J. Biol. Chem., 1933, 103, 471.
- Rosenbaum, J. D., and Lavietes, P. H., Lipoid-thiocyanate in serum. J. Biol. Chem., 1939, 131, 663.
- Bourdillon, J., and Lavietes, P. H., Observations on the fate of sodium sulfate injected intravenously in man. J. Clin. Invest., 1936, 15, 301.
- Haldane, J. S., and Priestley, J. G., Respiration. Yale University Press, New Haven, 1935. Second edition, pp. 387-399.
- Meakins, J. C., and Davies, H. W., Observations on the gases in human arterial and venous blood. J. Path. Bact., 1920, 23, 451.
- Collip, J. B., The action of HCO_3 ion and of morphine on the respiratory center. J. Physiol., 1920-21, 54, 58.
- Gesell, R., and Hertzman, A. B., The regulation of respiration. IV. Tissue acidity, blood acidity and pulmonary ventilation. A study of the effects of semipermeability of membranes and the buffering action of tissues with the continuous method of recording changes in acidity. Am. J. Physiol., 1926, 78, 610.
- Gesell, R., Krueger, H., Gorham, G., and Bernthal, T., The regulation of respiration. A study of the correlation of numerous factors of respiratory control following intravenous injection of sodium bicarbonate. Am. J. Physiol., 1930, 94, 387.
- Gollwitzer-Meier, K., Die chemische Atmungsregulation bei alkalischen Reaktion. Biochem. Ztschr., 1924, 151, 424.
- Ege, R., and Henriques, V., Untersuchung über die Bedeutung der Blutreaktion für die Lungenventilation. Biochem. Ztschr., 1926, 176, 441.
- Lowry, O. H., and Hastings, A. B., Personal communication.
- Shaw, L. A., and Messer, A. C., The transfer of bicarbonate between the blood and tissues caused by alterations of the carbon dioxide concentration in the lungs. Am. J. Physiol., 1932, 100, 122.
- Irving, L., Foster, H. C., and Ferguson, J. K. W., The carbon-dioxide dissociation curve of living mammalian muscle. J. Biol. Chem., 1932, 95, 95.
- Peters, J. P., Body Water, the Exchange of Fluids in Man. Charles C. Thomas, Springfield, Illinois, 1935.
- Roughton, F. J. W., Recent work on carbon dioxide transport by the blood. Physiol. Rev., 1935, 15, 241.
- Palmer, W. W., and Van Slyke, D. D., Studies of acidosis. IX. The relationship between alkali retention and alkali reserve in normal and pathological individuals. J. Biol. Chem., 1917, 32, 499.
- Hartmann, A. F., and Senn, M. J. E., Studies in the metabolism of sodium *r*-lactate. II. Response of human subjects with acidosis to the intravenous injection of sodium *r*-lactate. J. Clin. Invest., 1932, 11, 337.
- Jacobs, M. H., To what extent are the physiological effects of carbon dioxide due to hydrogen ions? Am. J. Physiol., 1920, 51, 321.

35. Jacobs, M. H., The production of intracellular acidity by neutral and alkaline solutions containing carbon dioxide. *Am. J. Physiol.*, 1920, 53, 456.
36. Gesell, R., On the chemical regulation of the respiration. I. The regulation of respiration with special reference to the metabolism of the respiratory center and the coördination of the dual function of hemoglobin. *Am. J. Physiol.*, 1923, 66, 5.
37. Gesell, R., Krueger, H., Nicholson, H., Brassfield, C., and Pelecovich, M., A comparison of the response of the anesthetized dog to lowered alveolar oxygen during uniform artificial ventilation and during normally controlled ventilation. *Am. J. Physiol.*, 1932, 100, 202.
38. Haldane, J. S., and Priestley, J. G., *Respiration*. Second Edition, Yale University Press, New Haven, 1935, pp. 99-100.
39. Winterstein, H., Die Reaktionstheorie der Atmungsregulation im Lichte neuerer Untersuchungen. *Klin. Wchnschr.*, 1928, 7, 241.
40. Nielson, M., Untersuchung über die Atemregulation beim Menschen. Besonders mit Hinblick auf die Art des chemischen Reizes. *Skandinav. Arch. f. Physiol.*, 1936, 74, 87, suppl. no. 10.
41. Schmidt, C. F., and Comroe, J. H., Jr., *Respiration*. *Ann. Rev. Physiol.*, 1941, 3, 151.
42. Laqueur, E., and Verzár, F., Über die spezifische Wirkung der Kohlensäure auf das Atemzentrum. *Pflügers Arch. f. d. ges. Physiol.*, 1911, 143, 395.
43. Campbell, J. A., Carbon dioxide tension and oxygen consumption during artificial respiration, acidosis and alkalosis. *J. Physiol.*, 1923, 57, 386.
44. Mellanby, J., The absence of relation between the amplitude of respiratory movement and the reaction of the blood. *J. Physiol.*, 1922, 56, 38P.
45. Atchley, D. W., Loeb, R. F., Richards, D. W., Benedict, E. M., and Driscoll, M. E., On diabetic acidosis. A detailed study of electrolyte balances following the withdrawal and reestablishment of insulin therapy. *J. Clin. Invest.*, 1933, 12, 297.
46. Kydd, D. M., Salt and water in the treatment of diabetic acidosis. *J. Clin. Invest.*, 1933, 12, 1169.
47. Stillman, E., Van Slyke, D. D., Cullen, G. E., and Fitz, R., Studies of acidosis. VI. The blood, urine, and alveolar air in diabetic acidosis. *J. Biol. Chem.*, 1917, 30, 405.
48. Stadie, W. C., Fat metabolism in diabetes mellitus. *J. Clin. Invest.*, 1940, 19, 843.
49. Harrison, H. C., and Long, C. N. H., The distribution of ketone bodies in tissues. *J. Biol. Chem.*, 1940, 133, 209.
50. Courtice, F. C., Douglas, C. G., and Priestley, J. G., Carbohydrate metabolism and muscular exercise. *Proc. Roy. Soc., London, Series B*, 1939, 127, 41.

EFFECT OF MUSCULAR EXERCISE UPON THE PERIPHERAL CIRCULATION IN PATIENTS WITH VALVULAR HEART DISEASE¹

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Considerable controversy still exists as to the hemodynamics associated with organic involvement of the valves of the heart in man. With respect to aortic insufficiency, Stewart (1), mainly on the basis of animal experiments, advanced the view that the collapsing pulse in this state is due to an increased blood flow through the capillaries following reflex inhibition of the vasomotor center, and not to regurgitation into the left ventricle. Hill and Rowlands (2), in order to explain the apparently greater blood pressure in the lower extremities as compared with that in the arms, postulated that the leg arteries are held in a contracted state, so that the brain might receive a sufficient supply of blood. However, Gladstone (3), also on theoretical grounds, came to the opposite conclusion, that the average rate of flow to the hand, arms, and kidneys is less than normal, while the legs receive a disproportionately large share of the total output of the heart.

In relation to the problem of hemodynamics in mitral stenosis, the experimental work has for the most part consisted of cardiac output studies. The results, however, have been contradictory, some of the investigators (4, 5) observing a decreased, and others (6, 7), a normal minute volume output in this state. With respect to the peripheral circulation, Meakins and his associates (4) reported a diminished oxygen saturation and an increased carbon dioxide content of venous blood from the arm. On the basis of these findings, they concluded that in this state the resting muscles in the extremities are partially deprived of their normal complement of arterial blood, in order that more essential organs might obtain an adequate supply of oxygen. According to them, some of the general symptoms observed in patients with mitral stenosis, such as cyanosis, weakness, and fatigue, are reflections of the diminished rate of peripheral blood flow. Stewart and his collaborators (8) studied the arm-to-tongue circula-

tion time and noted that the average velocity of blood flow was somewhat decreased in this state.

Since the above views are for the most part contradictory or based on theoretical considerations, it was thought worthwhile to study the actual rate of peripheral blood flow in aortic insufficiency and mitral disease by means of the venous occlusion plethysmographic method.

METHOD

The study was performed upon 29 ambulatory patients with aortic insufficiency, resulting from either rheumatic or syphilitic infection, and upon 16 patients with stenosis of the mitral valve of rheumatic origin. Subjects with such complicating factors as congestive heart failure or double valvular lesions were excluded. All of the patients with aortic insufficiency demonstrated a wide pulse pressure, the characteristic blowing diastolic murmur in the second intercostal space to the right of the sternum, and the left ventricular enlargement. In some, a capillary pulse was also present. In the case of the patients with mitral valvular disease, the typical presystolic murmur at the apex and the enlargement of the left auricle and bulging of the conus region (as demonstrated by fluoroscopy or x-ray) were always present.

Blood flow determinations, in cc. per minute per 100 cc. limb volume, were obtained separately in the hand, forearm, and leg, according to the technique previously described (9). The bath temperature (temperature of water in plethysmograph) was maintained at 32° C. and the room temperature between 25° and 27° C. The procedure generally followed consisted of placing a hand and a contralateral forearm or leg into plethysmographs and recording 15 to 20 resting control blood flow readings of the 2 extremities over a period of approximately 1 hour. Then, in the majority of patients, the local circulatory response to a specified amount of exercise was studied. With the forearm in the plethysmograph, the pressure in an air-filled 5 gallon bottle was raised to a definite level (60 to 70 mm. Hg) by means of ipsilateral manual compression of a blood pressure bulb. The work was performed in a period of approximately 1 minute. Immediately after the termination of the exercise, blood flow readings were taken at short intervals (every 10 or 15 seconds at first and later every 30 seconds) until the local circulation had returned to the control resting level. From these determinations, a graph was constructed, and by means of a planimeter the quantity of excess blood flow, over and beyond the amount which would ordinarily

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have entered the forearm at rest, was obtained (10). The figure was expressed as the blood flow repayment per 100 cc. limb volume. The same procedure was performed upon a series of 16 normal subjects, no attempt being made to eliminate the variable factor of physical fitness either in these or in the patients with valvular disease.

RESULTS

Examination of Table I reveals that for the most part the measurements of blood flow in the

TABLE I
Resting blood flow in aortic insufficiency

Subject	Age	Etiology	Blood flow in cc. per minute per 100 cc. limb volume			Blood pressure
			Hand	Forearm	Leg	
	<i>years</i>					
M. C.		Luetic	5.8		2.6	158/30-0
G. S.	37	Rheumatic		1.3	1.6	112/30-0
L. E.	52	Luetic	3.4	0.7	0.5	118/48
M. C.	48	Luetic	6.0	2.5		212/92
O. H.	33	Luetic	5.4	1.1		176/50
H. S.	22	Rheumatic	8.0	1.4		164/50
I. R.	35	Rheumatic	9.3	1.9	1.9	124/74
A. D.	55	Rheumatic	8.1	1.9	1.8	138/68
E. B.	59	Luetic	9.1	2.5	2.3	166/48-0
W. J.	56	Luetic	5.0	2.4	2.0	150/50
R. G.	41	Luetic		1.5	2.0	148/66-0
G. P.	55	Luetic		1.2	1.4	124/60
J. H.	38	Luetic		1.3	1.5	110/20-0
S. M.	65	Luetic		1.8	1.8	126/46
J. D.	44	Luetic		1.2		128/82
P. M.	55	Luetic		2.0	1.2	194/76
L. W.	70	Luetic		3.1	1.7	212/40-0
D. P.	63	Luetic		1.7	2.7	180/82
W. H.	47	Luetic		1.5	2.0	108/30-0
E. A.	56	Luetic			1.6	230/50
C. S.	59	Luetic		2.1	1.5	116/36
S. D.	42	Luetic		1.2	0.8	136/20-0
J. L.	63	Luetic		0.8	0.7	164/40-0
S. R.	55	Luetic		1.0	0.9	124/58
P. S.	21	Luetic		1.3	1.0	110/30-0
E. R.	53	Luetic		7.9	3.6	198/80-0
R. M.	15	Rheumatic		5.8	3.0	160/0
C. J.	24	Rheumatic		1.8	1.2	130/70
R. S.	19	Rheumatic		1.2	2.1	108/64

hand in the patients with aortic insufficiency were either in the lower range of the readings for the normal group or definitely below it (the average being 6.7 cc. per minute per 100 cc. limb volume, as compared with 9.3 cc. (σ —2.1) for the control series).² In the forearm, of the 27 patients studied, 21 demonstrated blood flow measurements which fell within the range of the normal series (1.8 cc. per minute per 100 cc. limb volume (σ —0.7)), while 3 showed somewhat diminished

and 3, significantly increased readings. The average for the whole group was 2.0 cc. per minute per 100 cc. limb volume. In the leg, 16 patients demonstrated blood flow measurements which were within the range of the normal series (1.4 cc. per minute per 100 cc. limb volume (σ —0.5)), while 3 showed somewhat diminished and 6, significantly increased readings. The average for the whole group was 1.7 cc. per minute per 100 cc. limb volume.

TABLE II

Resting blood flow in mitral valvular disease

Subject	Age	Blood flow in cc. per minute per 100 cc. limb volume		Blood pressure	Circulation time
		Hand	Forearm		
	<i>years</i>				<i>seconds</i>
R. S.	28	7.2	1.0	104/68	
J. D.	38	5.2	1.6	120/70	16
H. S.	27	4.8	0.9	114/80	
W. J.	17	6.0	1.5	98/56	15
E. L.	35	8.0	1.5	110/64	
G. E.	13	7.6	2.8	120/82	9
A. T.	61	2.1	1.1	128/78	
M. S.	46	6.4	1.9	106/70	11
M. M.	35	7.2	3.5	124/70	
B. L.	43	8.2	1.1	110/80	
S. N.	33	9.0	1.9	100/58	
D. R.	18		1.3	114/70	
F. B.	14		2.5	104/62	
J. Y.	15		1.2	116/66	
J. C.	16		1.3	130/80	
L. C.	16		1.7		

Table II reveals that the rate of resting blood flow in the hand in the patients with mitral valvular disease also fell, either within the lower range of that obtained for normal subjects, or somewhat below it (an average of 6.5 cc. per minute per 100 cc. limb volume, as compared with an average control of 9.3 cc. (σ —2.1)). In the forearm, the readings were, for the most part, similar to those of the normal series (an average of 1.7 cc. as compared with 1.8 cc. (σ —0.7)). In only occasional cases did the determinations fall beyond the control range.

In respect to the response to a given quantity of work, in 10 of the 14 patients with aortic insufficiency, the excess blood flow elicited by the exercise was definitely greater than the average reading of 37.8 cc. per 100 cc. of limb volume for the control group, while in 4, the response was the same or less than this figure (Table III). It is

² For controls, the results obtained in a series of 90 normal subjects, previously reported (11), were used.

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TABLE III
Response of the forearm to exercise in aortic insufficiency

Subject	Age	Post-exercise repayment	Subject	Age	Post-exercise repayment
	years			years	
S. R.....	55	31.1	E. B.....	59	67.9
C. S.....	59	67.6	P. S.....	21	60.7
W. J.....	56	42.7	G. P.....	55	33.8
R. G.....	41	78.1	R. S.....	19	42.6
W. H.....	47	49.5	C. J.....	24	58.0
D. P.....	63	55.8	S. M.....	65	114.5
J. D.....	44	30.8	J. H.....	38	24.7

Pressure in 5-gallon bottle raised to 60 to 70 mm. Hg by means of compression of a blood pressure bulb, 55 to 60 times, in approximately 1 minute.
Repayment—Quantity of excess blood flow for each 100 cc. limb volume, entering the extremity in post-exercise period.

of interest that in 3 of the latter subjects (G. P., J. D., and S. R.) there did not appear to be much disturbance of the hemodynamics, the diastolic pressure in each instance being within the normal range (Tables I and III).

TABLE IV
Response of the forearm to exercise in mitral valvular disease

Subject	Age	Post-exercise repayment	Subject	Age	Post-exercise repayment
	years			years	
M. S.....	46	60.1	D. R.....	18	47.7
J. C.....	16	58.2	F. B.....	14	66.4
G. E.....	13	55.2	J. Y.....	15	53.8
M. M.....	35	55.8	S. N.....	33	32.6

Pressure in 5-gallon bottle raised to 60 to 70 mm. Hg by means of compression of a blood pressure bulb, 55 to 60 times in approximately 1 minute.
Repayment—Quantity of excess blood flow for each 100 cc. limb volume, entering extremity in post-exercise period.

The blood flow responses to exercise in the patients with mitral valvular disease were essentially similar to those reported above. In 7 of the patients, the excess blood flow elicited by the work was definitely greater than the average of 37.8 cc. per 100 cc. limb volume for the control subjects, while in 1, the response was less than normal (Table IV).

DISCUSSION

From the foregoing, it would appear that in the majority of patients with compensated aortic

insufficiency and mitral valvular disease, the average rate of blood flow in the forearm or leg was not significantly altered. The increased or decreased peripheral circulation in some of the subjects in the two series could not, in any way, be correlated with the other objective signs, and no explanation can be offered for these exceptions. The results in the hand have not been given much significance, since it has been shown that the blood vessels in this site react markedly to all types of vasoconstricting stimuli; the vascular responses in the forearm and leg being much more representative of the peripheral circulation generally (12). With respect to aortic insufficiency, therefore, the findings, as a whole, would be in accord with the view that the peripheral resistance in the extremities is not materially altered in this state. In the case of mitral stenosis, the results do not support the conclusions of Meakins and his associates (4) that there is a shunting of blood from the extremities to other portions of the body in this condition, with a consequent diminution in peripheral circulation.

The blood flow repayment to exercise, however, was generally greater in the patients with valvular disease than in normal subjects, despite the fact that the amount of work performed was not sufficient to tax the compensatory mechanisms severely. Before discussing the significance of this finding, it is necessary to explain briefly the rationale for the procedure used. This is based on the assumption that, if the augmented circulation present during work is insufficient to meet entirely the increased demands of the tissues, a blood flow debt must be incurred; this, in turn, being repaid in the subsequent period of rest. The magnitude of the blood flow repayment would thus serve as an index of the efficiency of the compensatory response during exercise (10). It would appear from the data collected that in most of our patients with valvular disease the compensatory mechanisms, ordinarily elicited by the stimulus of work, were not as adequate as in normal subjects, and hence the blood flow repayment in the post-exercise period was necessarily of greater magnitude. Another possibility is that these patients utilized a greater amount of energy in the performance of the work and thus incurred a greater blood flow debt. It is probable that both

mechanisms were responsible. The results with aortic insufficiency, therefore, do not support the view of Jokl and Suzman (13), who consider this state to be compatible with little or even practically no functional impairment. With respect to the response to exercise in mitral stenosis, Dennig and Prodger (14) and Nielsen (7) have expressed the opinion that the cardiac output under these conditions does not increase in accordance with the greater oxygen demands, and hence the amount of work which will produce fatigue is much less than that in normal subjects. Our findings in reference to the peripheral circulation help to support this view.

SUMMARY AND CONCLUSIONS

Using the venous occlusion plethysmographic method, the rate of resting peripheral blood flow and the circulatory response to exercise were studied in a series of 29 patients with insufficiency of the aortic semilunar valves, and in 16 subjects with mitral valvular disease.

The average circulation in the hand was found to be somewhat diminished in both series of patients as compared with that for the control series, while the readings in the forearm and leg in the majority of the cases fell within the normal range.

The post-exercise response of the blood vessels in the forearm to a specified amount of work was generally greater than that in the control group.

It was concluded that, in the majority of the patients with aortic insufficiency or mitral valvular disease, no evidence was found to indicate that excessive vasodilatation or vasoconstriction exists in the vessels of the forearm or leg.

On the basis of the results obtained with a period of exercise, it appears that either the compensatory circulatory mechanisms elicited by such a stimulus are not as effective as normal, that the work is performed with less efficiency, or possibly that both mechanisms are operating in this condition.

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BIBLIOGRAPHY

1. Stewart, H. A., Experimental and clinical investigation of the pulse and blood pressure changes in aortic insufficiency. *Arch. Int. Med.*, 1908, 1, 102.
2. Hill, L., and Rowlands, R. A., Systolic blood pressure; (1) in change of posture; (2) in cases of aortic regurgitation. *Heart*, 1911-12, 3, 219.
3. Gladstone, S. A., Few observations on haemodynamics of normal circulation and changes which occur in aortic insufficiency. *Bull. Johns Hopkins Hosp.*, 1929, 44, 83.
4. Meakins, J., Dautrebande, L., and Fetter, W. J., The influence of circulatory disturbances on the gaseous exchange of the blood; blood gases and circulation rate in cases of mitral stenosis. *Heart*, 1923, 10, 153.
5. Kerkhof, A. C., Minute volume determinations in mitral stenosis during auricular fibrillation and after restoration of normal rhythm. *Am. Heart J.*, 1936, 11, 206.
6. Ewig, W., and Hinsberg, K., Kreislaufstudien, II. *Ztschr. f. klin. Med.*, 1931, 115, 693.
7. Nielsen, H. E., Clinical investigations into the cardiac output of patients with compensated heart disease during rest and during muscular work. *Acta med. Scandinav.*, 1937, 91, 223.
8. Stewart, H. J., Deitrick, J. E., Watson, R. F., Wheeler, C. H., and Crane, N. F., The effect of valvular heart disease on the dynamics of the circulation; observations before, during and after occurrence of heart failure. *Am. Heart J.*, 1938, 16, 477.
- 9a. Abramson, D. I., Zazeela, H., and Marrus, J., Plethysmographic studies of peripheral blood flow in man. I. Criteria for obtaining accurate plethysmographic data. *Am. Heart J.*, 1939, 17, 194.
- b. Ferris, E. B., Jr., and Abramson, D. I., Description of a new plethysmograph. *Ibid.*, 1940, 19, 233.
10. Abramson, D. I., and Fierst, S. M., Peripheral vascular response to exercise in the hyperthyroid state. *J. Clin. Invest.*, 1941, 20, 517.
11. Abramson, D. I., and Fierst, S. M., Resting blood flow and peripheral vascular responses in hypertensive subjects. *Am. Heart J.*, 1942, 23, 84.
12. Abramson, D. I., and Ferris, E. B., Jr., Responses of blood vessels in the resting hand and forearm to various stimuli. *Am. Heart J.*, 1940, 19, 541.
13. Jokl, E., and Suzman, M. M., Aortic regurgitation and mitral stenosis in a Marathon runner, with special reference to effects of valvular heart disease on physical efficiency. *J. A. M. A.*, 1940, 114, 467.
14. Dennig, H., and Prodger, S. H., Herzkrankhe bei Arbeit. *Deutsches Arch. f. klin. Med.*, 1933, 175, 170.

THE QUALITATIVE EXAMINATION OF URINARY CALCULI

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Heller's (1) monograph, published in 1860, contains a scheme for the chemical examination of urinary calculi which has succeeded in holding the field for 80 years. This scheme is still reproduced in standard textbooks and in general use in many clinics, if we may judge from the periodic literature (2, 3).

Heller and his successors depended largely upon the use of mechanical means to separate the different constituents of calculi, before proceeding to carry out their chemical identification. Such a method is doubtless serviceable in dealing with large bladder stones, but is difficult to apply to small calculi of renal and ureteral origin such as those which make up the bulk of specimens in a modern clinic. The method is not well adapted to the study of stones which contain intimate mixtures of certain constituents.

During the present century, analytical procedures have been improved and some of the elementary facts at the basis of stone formation have been discovered, so that Heller's scheme is no longer capable of satisfying present-day requirements. In recent years, a few articles have appeared, suggesting alternative procedures (4 to 9), some of which are quantitative (10 to 13). To judge the adequacy of these analytical schemes, we may set up the following criteria:

1. The tests used must be selected with regard to their simplicity and accuracy, and should be applicable to small amounts of material.
2. The scheme must be capable of analysing the mixtures which experience teaches us are likely to be found in naturally-occurring stones.
3. The results must be at least roughly quantitative, if the specimen is to be adequately classified.

The analytical schemes in the literature meet these criteria in varying degrees. Curiously enough, many of the authors, like Heller, make the assumption that they are dealing with a single constituent, a simplification which is far from warranted and which may obviously lead to erroneous conclusions.

In the present scheme, the object has been to test for the presence of every common constituent of urinary stones, so that each will be identified if present in significant amounts. Where possible, the various constituents have been removed in consecutive steps, so that the chance of interference has been minimized. A sample of 5 to 10 mgm. is adequate, although it is generally possible to obtain a useful result from as little as 1 to 2 mgm.

The reagents are conveniently kept in 30 ml. dropping bottles with ground-in pipettes and nipples. The tests are carried out in 15 ml. centrifuge tubes, in volumes of 0.2 to 0.5 ml. In this way, consecutive separations may easily be made by use of the centrifuge.

REAGENTS

Hydrochloric Acid, Concentrated.

Nitric Acid, Concentrated.

Ammonium Hydroxide, Concentrated.

3 per cent Ammonium Molybdate.

Nessler's Reagent.

Sodium Acetate, Saturated Solution.

2 M Ammonium Chloride—10.7 grams NH_4Cl dissolved in 100 ml. of water.

0.4 M Oxalic Acid—5.0 grams $\text{C}_2\text{H}_2\text{O}_4$, 2 H_2O in 100 ml. of water.

1 M Disodium Hydrogen Phosphate—Dissolve 35.8 grams Na_2HPO_4 , 12 H_2O in 100 ml. of water.

1 N Sodium Hydroxide.

5 per cent Sodium Cyanide.

Sodium Nitro-prusside Reagent. Dissolve 10 grams of the solid in 100 ml. of distilled water, adding 2 ml. of concentrated sulphuric acid.

Diazo Reagent. This must be freshly prepared by adding 0.3 ml. of 0.1 per cent *Sodium Nitrite* to 10 ml. of 0.1 per cent *Sulphanilic Acid*, which has been made up in a solution containing 15 ml. of concentrated *Hydrochloric Acid* per litre.

PROCEDURE

The stone is weighed, measured, and examined with the hand lens. Adherent blood is removed as far as possible. If necessary, the stone is sectioned to determine its homogeneity. One or more samples are taken, depending on the uniformity of the stone, and reduced to powder in a small mortar.

1. Ignite some of the powder. Cystine, fat or wax, and blood clots will burn with a flame, if present in sufficient amounts. The purines glow without flame production. If no ash remains, it is unnecessary to carry out the tests for inorganic constituents, except for ammonia.

2. *Uric acid.* Every sample should be tested for the presence of uric acid. The well-known murexide test is satisfactory. One milligram or more of the powder is treated with 2 or 3 drops of concentrated HNO_3 in a porcelain dish. The mixture is carefully evaporated to dryness, and heating continued until the colour change is complete. If the test is carried out on the water bath, uric acid gives first a yellow colour, which may be mistaken for the reaction for xanthine. If heating is continued a few minutes longer, xanthine shows no further alteration, but in the case of uric acid the colour changes to orange and finally to scarlet. On addition of a drop of ammonia, the uric acid oxidation product assumes a brilliant purple colour, while xanthine changes to orange, which becomes a red on further heating.

Since uric acid may occur as ammonium urate as well as the free acid, the test for ammonia should be made. The powder is extracted with hot dilute HCl and the extract tested for ammonia as in Step 9.

3. *Xanthine.* This substance may be identified as described in the previous paragraph. On account of its extreme rarity, a positive nitric acid test calls for confirmation. Ehrlich's diazo test may be used since uric acid is the commonest substance which might be mistaken for xanthine. A little of the powder is boiled with 1 to 2 ml. of freshly-prepared diazo reagent, and a few drops of Normal NaOH are added. Xanthine couples with the reagent to produce a brilliant red wine colour. Uric acid gives no colour or a pale yellow, depending on the purity of the specimen. The test is not specific; it is given by many substances including the purines which are not substituted at positions 7 and 8. Its use, however, assures that uric acid will not be mistaken for xanthine. The test might serve to detect xanthine in the presence of uric acid.

4. *Cystine.* A pinch of the powder is boiled with 2 to 3 ml. of water, then treated with an equal amount of 5 per cent NaCN solution. After 5 minutes, a few drops of sodium nitro-prusside solution are added. Cystine is indicated by a beautiful magenta colour. This test, described by Brand (14), is much quicker than the older method of recrystallizing from ammonia, and identifying the hexagonal crystals under the microscope. The solubility of the specimen in NH_4OH and in dilute HCl should be tested as a rapid means of confirmation, and as a test of its purity, since no other common constituent of calculi is soluble in both. Cystine stones, as a rule, contain only small traces of other substances, and for ordinary purposes these do not need to be further identified.

5. *Microscopic examination.* This step is conveniently carried out at this time, in conjunction with Step 6. A little of the powder is mounted in water under a cover slip, and examined under the microscope. This gives added information as to the homogeneity of the speci-

men, and may afford a clue as to its composition. The "triple phosphate" crystals of ammonium magnesium phosphate are sometimes easily recognized. Calcium oxalate may often be recognized under the hand lens as sharp, knife-edged crystals; when powdered, the crystals fracture irregularly, so that under the microscope the fragments resemble broken glass. Though calcium carbonate crystals may sometimes be recognized, other calcium salts are often amorphous or difficult to identify.

6. *Test for carbonate.* A little of the powder is placed on a glass slide and intimately mixed with a drop of water, to expel air bubbles. A cover slip, carrying a small drop of concentrated HCl is then inverted upon the powder. The evolution of CO_2 can be readily recognized under the microscope.

Aside from this test for the identification of carbonate, which has been described by Newcomb (11), the behaviour of the powder after the addition of acid is often characteristic, and sufficient to indicate its composition. Phosphates and carbonates go rapidly into solution, oxalates dissolve slowly, while uric acid is insoluble. Cystine, on contact with concentrated HCl , undergoes an instantaneous recrystallization which has been described by Wollaston (15).

7. *Test for phosphate.* A small amount—roughly 2 to 3 mgm.—of the powder is dissolved in 3 or 4 drops of concentrated HNO_3 , by boiling in a test tube. The solution is transferred to a centrifuge tube¹ and 3 or 4 drops of 3 per cent ammonium molybdate solution are added. A yellow precipitate indicates the presence of phosphate. This reaction may be slow, so that it is sometimes necessary to allow the tube to stand an hour or more before precipitation is complete. If proteins are present, they are thrown down by the reagent with formation of a white flocculent precipitate.

Experience has shown that tests carried out in HNO_3 solution as described above are more sensitive than similar tests carried out in HCl solution. When dealing with stones which contain large amounts of phosphate it is more convenient to use a portion of the HCl solution prepared in Step 8. When small amounts of phosphate are to be detected, however, such as are often present in calcium oxalate stones, HNO_3 solution is to be preferred, since HCl tends to increase the solubility of the phosphomolybdate.

8. *Separation of acid-insoluble material.* About 5 to 10 mgm. of the powder are boiled in a test tube with 1 to 2 drops of concentrated HCl and a little water, until the inorganic constituents have gone into solution. The solution is transferred to a centrifuge tube, diluted to about 0.7 ml., and insoluble residue is spun down. The supernatant fluid is removed with a dropping pipette and divided into two parts, or into three, if this solution is to be tested for phosphate. The insoluble residue, if any, is then examined under the microscope. It will usually consist of cellular and other protein debris. Uric acid may be present, as well as yellow-coloured hemo-

¹ The powder may be dissolved by boiling in a Pyrex centrifuge tube, in order to avoid the necessity of transfer.

globin derivatives. In stones from herbivorous animals, silica may be found.

If the supernatant is cloudy, it may be necessary to filter with suction through a small filter paper, about 1 cm. in diameter, supported by a filter plate. The filter should be washed with a few drops of water to avoid loss of the solutes. The clear fluid obtained by centrifuging or filtration is known as Solution 1.

9. *Test for ammonia.* To part of Solution 1, a few drops of Nessler's solution are added, sufficient to make the mixture definitely alkaline. The formation of a white precipitate is disregarded. An orange colour or precipitate indicates the presence of ammonia. Formalin interferes with this test.

10. *Test for calcium oxalate.* To another part of Solution 1, add a few drops of saturated sodium acetate, until the acidity of the solution is brought to pH 5 approximately, as indicated by Hydrion paper.² If calcium oxalate is present it will be precipitated almost quantitatively. The solution is mixed, permitted to stand for 15 minutes to allow complete precipitation, and the calcium oxalate thrown down in the centrifuge. The supernatant fluid (Solution 2) is transferred to another centrifuge tube.

This test for calcium oxalate is essentially that of Domanski (5). In our experience, the microscopic examination of the precipitate, and the permanganate test for confirmation are unnecessary refinements.

11. *Test for non-oxalate calcium.* To Solution 2, a drop of ammonium chloride is added, to prevent the precipitation of magnesium salts. Then 2 to 4 drops of oxalic acid solution are added, or sufficient to precipitate calcium which has not been precipitated in Step 10. Calcium, originally present as carbonate, phosphate, or citrate, is thrown down at this stage. The mixture is stirred, allowed to stand for 15 minutes, and centrifuged as before. The supernatant (Solution 3) is transferred to another centrifuge tube.

12. *Test for magnesium.* To Solution 3, sufficient ammonium hydroxide is added to bring the pH to about 8, by Hydrion paper.³ In order to make the reaction more sensitive, 1 or 2 drops of disodium hydrogen phosphate are also added. A crystalline precipitate indicates the presence of magnesium. The star-shaped crystals which form should be checked by microscopic examination. Carried out in this way, the test is sufficiently sensitive to detect the magnesium present in a small sample of bone.

² Those who prefer the use of a liquid indicator may add a drop of 0.016 per cent solution of brom cresol green, then add the acetate solution until the color changes from yellow to green. In order to avoid increasing the volume it may be advantageous to place the drop on a white porcelain tile and use it as an external indicator.

³ A 0.5 per cent solution of phenolphthalein in 50 per cent alcohol may be used as an indicator for this step, if preferred.

DISCUSSION

The method described above has certain novel features. Among these is the separation of the calcium into oxalate and non-oxalate fractions. Here the question of the qualitative detection of non-oxalate calcium is not the essential point, as experience has shown that it is present to some extent in practically every inorganic stone. On the other hand, it is sometimes important to have some idea of the relative amounts of these calcium fractions. If the separation is made carefully, in a semi-quantitative way, the volumes of the precipitates may be compared after centrifuging. One can then decide by inspection whether one is dealing with a stone which is predominantly composed of calcium oxalate, or whether oxalate is present in traces in a stone of fundamentally different nature.

A more important feature of the scheme is the determination of the presence or absence of magnesium. Strangely enough, it has been customary to neglect this step in qualitative examination of calculi. For instance, Domanski (5) omits the differentiation between phosphates, and states that if phosphate is present it is legitimate to assume the presence of calcium and magnesium phosphate. In our experience, this is not the case. There is an important group of phosphate stones which do not contain magnesium, as will be shown in the following paper.

Finally, if we allow ourselves to make certain assumptions which are commonly made about the composition of inorganic stones, the method permits certain checks on the accuracy of the results.

Thus, if magnesium is absent, carbonate or phosphate should be accompanied by non-oxalate calcium in at least equivalent amounts. Similarly, since ammonia probably means either ammonium urate or ammonium magnesium phosphate, if the former is absent the ammonia should correspond to the magnesium, at least in the case of stones caused by urea-splitting organisms. Experience tends to show that these rules are qualitatively valid, in an approximate way, for human material.

During the four years in which the method has been in routine use in this laboratory, no difficulties have arisen in its application. It is necessary to caution a technician using it for the first time against the use of too much of the powdered stone

for the analysis, or using the concentrated reagents too freely, as salts may be formed in amounts exceeding their solubilities and so give rise to unexpected precipitates.

One might imagine cystine being mistaken for calcium oxalate in Steps 8 and 10, as it would go into solution in dilute acid, and unless a very small specimen were taken, might precipitate on addition of sodium acetate. In such case, no confusion should arise if ignition has been carried out beforehand in proper sequence. Wollaston's crystals will also be recognized in Step 6. In actual work, no difficulty has been experienced on this score.

A more serious problem is the identification of citrate, which has been reported in experimental rat calculi, but has not been found in significant amounts in human stones (16). There does not seem to be any test for the qualitative identification of citrate, suitable for routine use in the analysis of urinary calculi.

SUMMARY

1. A method for the qualitative chemical examination of urinary calculi is described.

2. The distinctive features of the method include the separation of the calcium into oxalate and non-oxalate fractions, and the determination of the presence or absence of magnesium.

BIBLIOGRAPHY

1. Heller, J. F., *Die Harnconcretionen, ihre Entstehung, Erkennung, und Analyse mit besonderer Rücksicht auf Diagnose und Therapie der Nieren- und Blasenkrankung*. Wein, Tendler u. Comp., 1860.
2. Randall, A., Campbell, E. W., and Beeson, H. C., A simple method of chemical analysis of urinary

- calculi with a report of the chemical composition of a recent series. *Urol. and Cutan. Rev.*, 1934, 38, 29.
3. Keyser, L. D., Recurrent urolithiasis: etiologic factors and clinical management. *J. A. M. A.*, 1935, 104, 1299.
4. Ranganathan, S., Chemical composition of urinary calculi in rats. *Indian J. M. Research*, 1930, 18, 599.
5. Domanski, T. J., Renal calculi: a new method for qualitative analysis. *J. Urol.*, 1937, 37, 399.
6. Domanski, T. J., Analysis of urinary calculi. *Am. J. Clin. Path., Tech. Suppl.*, 1938, 2, 157.
7. Kamlet, J., Analysis of renal calculi. *J. Lab. and Clin. Med.*, 1937, 23, 321.
8. Higgins, C. C., and Mendenhall, E. E., Factors associated with recurrent formation of renal lithiasis with report of new method for qualitative analysis of urinary calculi. *J. Urol.*, 1939, 42, 436.
9. Seifter, J., and Trattner, H. R., Simplified qualitative analysis of urinary calculi by spot tests. *J. Urol.*, 1939, 42, 452.
10. Newcomb, C., and McCarrison, R., The composition of vesical calculi. *Indian J. M. Research*, 1928-29, 16, 1036.
11. Newcomb, C., A scheme for the analysis of small urinary calculi. *Indian J. M. Research*, 1930, 17, 735.
12. Kya, L. T., The composition of vesical calculi. *Chinese M. J.*, 1936, 50, 797.
13. Brown, H., Micromethods for the quantitative analysis of urinary calculi. *J. Lab. and Clin. Med.*, 1939, 24, 976.
14. Brand, E., Harris, M. H., and Biloon, S., Cystinuria. The excretion of a cystine complex which decomposes in the urine with the liberation of free cystine. *J. Biol. Chem.*, 1930, 86, 315.
15. Wollaston, W. H., On cystic oxide, a new species of urinary calculus. *Proc. Roy. Soc., London*, 1810, 100, 223.
16. Schneider, H., and Steenbock, H., Calcium citrate uroliths on a low phosphorus diet. *J. Urol.*, 1940, 43, 339.

THE CLASSIFICATION AND CHEMICAL PATHOGENESIS OF URINARY CALCULI

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Classifications of urinary calculi are of two sorts, simple and complex. Wollaston's (1) scheme (1810), will serve as an example of the former. He named 6 classes of stones: (1) uric acid; (2) oxalate of lime; (3) phosphate of lime; (4) ammoniacal phosphate of magnesia; (5) fusible calculus (a mixture of the last two); and (6) cystic oxide (cystine). One may observe that Wollaston named only one mixture in his scheme, and so solved the question of classifying mixed stones by neglecting it. Heller's (2) classification (1860), which has been reproduced in countless textbooks and profoundly influenced the literature of the subject, is also one of the simple sort. It mentions 8 classes, some of them very rare or unimportant. The classifications used by numerous surgical authors who have treated the question of calculi in text-books or journal articles are often of the simple type.

The tendency among analysts has been towards complex classifications. These are likely to list not only the simple chemical constituents but also the various mixtures which have been observed. A recent classification of this type is that of Domanski (3), which makes use of 13 headings.

It has become customary to speak of the stones as composed of carbonates, phosphates, or oxalates, so that attention is focussed upon the acid radicals, rather than upon the salts as a whole or upon the metallic content. This tendency has had two unfavourable consequences. Attention has been directed away from the metallic elements, which happen to have far more physiological significance; and in the case of phosphates, as we shall see, these stones have often been lumped in a single group, which, on the grounds of pathogenesis, should certainly be subdivided. Hence, while many tables of composition in the literature contain irrelevant detail, others omit facts of etiologic significance.

The present paper presents a classification based

upon the chemical pathogenesis underlying stone formation and evidence supporting the clinical value of chemical analysis of urinary calculi. For the purposes of this article, it is unnecessary to consider anatomical defects, developmental or acquired, or pathologic processes, such as the papillary plaques described by Randall (4), although these may often have an important place in the pathogenesis of stones. It is generally recognized that an organic "matrix" can be demonstrated in most stones, but whether this "matrix" is "primary" or "secondary" is beside the point. The chemical mechanisms which concern us do not necessarily have to do with the earliest origin of a concretion, but rather with this question: Granted that a calculus has had its origin, what are the conditions which allow urinary solutes to continue to be deposited upon its surface? A widely accepted view is the assumption that there is a "protective colloid mechanism," which ordinarily prevents precipitation of sparingly soluble substances from the urine. Derangement of this mechanism is supposed to permit the deposition of such substances as are present in excess of their solubility. Without examining this hypothesis in detail, it is fair to say that there is little direct evidence in its favour, and that the weight of experimental proof is against it (5, 6). The results of the present study lend it no support, for we do not find mixed stones containing all possible stone-forming constituents, nor miscellaneous mixtures, such as the "protective colloid" theory would lead one to expect. We find, as a matter of fact, a limited number of combinations, not more than four or five, each of which would appear to depend upon a chemical mechanism of its own.

The solubility in urine of any stone-forming substance is not a simple matter, since this solubility may deviate widely from its solubility in pure water, depending upon the composition of the urine. It has been shown that urinary salts

have a marked effect upon the solubility of one another (7), and that complex formations sometimes occur, leading to very considerable increases in solubility. Quantitative studies of these effects have been made by Hammarsten (8) and Medes (9). There is no reason to doubt that factors of this sort influence the amount of stone-forming substances which the urine is able to hold in solution, and that they may frequently determine whether a calculus will form and grow, or tend to regress. Hammarsten has demonstrated the importance of solubility effects in determining the genesis of experimental calculi in the rat (6), and in influencing their regression (10), with special reference to the importance of magnesium in the case of calcium oxalate stones. In dealing with the general question of solubilities, the importance of urinary pH is well known.

Having briefly mentioned the principles which limit solubility in normal urine, we may now consider those factors which determine whether these limits will be exceeded or not. Aside from the matter of urine volume, these are two in number, diet and intermediary metabolism. It will be worth while to examine each of these in relation to each type of stone.

Uric acid stones. These stones usually consist of the free acid and are formed in acid urine. It is generally agreed that a strongly acid urine is the most important factor in their genesis—a belief supported by clinical experience (11) and experimental work (12). The likelihood of such stones may be increased as a result of excessive purine in the diet, as shown by Hammarsten's (12) experiments, or the uric acid may be of endogenous origin, as in cases of gout (11) and of leukemia (13).

The mechanism underlying the formation of ammonium urate stones is not at all clear. Hammarsten (14) is of the opinion that they may be formed independently of infection.

Cystine stones. These are only to be found in cases of cystinuria, and are the best example of a type of stone due to a derangement of intermediary metabolism. In a well-marked case, the urine may contain 0.4 to 1.0 grams of cystine per day. Although part of this cystine is considered to be endogenous in origin, there is reason to believe that it is mainly exogenous and derived from dietary methionine (15). If the urine is rendered

strongly alkaline, the cystine may sometimes be kept in solution (16).

Xanthine stones. These stones are so rare in the human that little or nothing is known of the mechanisms underlying their formation. There is doubtless some derangement of purine metabolism, leading to excessive excretion of this substance in the urine.

Primary calcium stones. This term may be used to designate the large group of stones composed of calcium salts which are not the direct result of urinary tract infection. This group is an important one, making up more than half the total number of cases in many communities. The stones are composed of the calcium salts of oxalic, phosphoric, carbonic, and perhaps citric and other organic acids. A review of the literature leaves one with the impression that the grouping is a valid one, since the element calcium appears to play the chief rôle, to which the rôle of the acid radicals is subsidiary. There seems to be some reason to think that excessive urinary excretion of calcium is an important factor in the group as a whole. Such excess may have an extrinsic cause, such as diet or medication, and strong evidence has been adduced in favour of the hypothesis. On the other hand, the hyperexcretion may arise from internal disease or metabolic error, such as hyperparathyroidism. Although it is suspected that such an excess is common, we do not know how often primary calcium calculi are accompanied by excessive urinary excretion of calcium in clinical cases.

The literature of experimental calcium lithiasis is too complex and extensive to permit a summary to be brief and at the same time adequate. Avitaminosis, especially a partial avitaminosis A has led to a high incidence of stone in the hands of most investigators (17 to 19). Hypervitaminosis D has also been successful, alone (20), and in combination with avitaminosis A (21). There seems to be good evidence that excessive calcium in the diet may be a factor in stone production (22, 23), although some investigators (24) prefer to regard an imbalance of the Ca:P ratio as the cause (24). Paradoxically, insufficient calcium in the diet may lead to greatly increased excretion of calcium in the urine; low magnesium intake also tends to cause a negative calcium balance, and if both elements are deficient in the

diet, Hammarsten has observed summation of these effects (6, 10). These experiments are of the greatest interest, for she found that if the diet yielded a low urine pH, calcium oxalate stones were formed. With an increase in pH, she found an increase in calcium phosphate calculi, and she pointed out that others had found phosphate, carbonate, and possibly citrate stones, when urine pH deviated towards the alkaline side. From these experiments, it would appear that hypercalciuria, however produced, is the determining factor in the production of calcium stones, while the acid radical with which it is combined is largely dependent upon the pH of the urine.

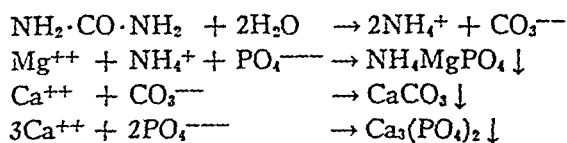
The relationship between hyperparathyroidism and calculi has been well studied (25 to 27). The decalcification of bone, the hypercalcemia, and the excessive excretion of calcium in the urine are well established. Excessive excretion of phosphate also occurs (28). As pointed out by Albright, Aub and Bauer (29), these factors favor the formation of calcium phosphate stones. But Hunter and Turnbull (25) found carbonate present, while Barney and Jones (30) and Albright, Sulkowitch and Chute (31) have reported calcium oxalate stones. While these authors have not correlated the type of stone with the pH of the urine in these cases, the analogy to Hammarsten's experimental work is unmistakable.

Excessive urinary excretion of calcium may also occur following disease and injury of bone, and as a result of rarefaction incidental to bed rest and fixation of the limbs (32). Cases of this sort sometimes suffer from stones in the kidney pelvis. As the stones are radio-opaque and may arise independently of infection, they are presumably primary calcium stones. When these patients became ambulatory, the stones frequently disappear. This sequel is probably accompanied by a striking decrease in urinary calcium and by recalcification of the skeleton. Other causes of excessive excretion of calcium in the urine include hypervitaminosis D, hyperthyroidism, and acidosis. These factors are less definitely linked to urinary lithiasis. Cushing's syndrome should also be mentioned.

Flocks (33) has recently called attention to excessive calcium excretion in the urine of patients suffering from calcium urolithiasis. He attributed two of his cases to hyperparathyroidism, others to intrinsic renal changes. No explanation

could be found for the remainder. It seems unlikely that the excessive urinary calcium could be due to a dietetic cause, since control cases on the same diets failed to show excessive excretion. Organisms were found in the urines of all patients, some of them urea-splitting types. Hence some of the calculi may not have been primary calcium stones. His results have not been confirmed. But, even if they prove to be misleading, it is certain that numerous human cases are to be observed in which primary calcium stones are associated with excessive urinary excretion of calcium. Can such stones arise when calcium excretion is normal? Although specific proof by quantitative urine studies is lacking, general clinical experience would suggest that they can. If so, the factors which influence the solubility of sparingly soluble calcium salts in urine assume great importance (7 to 9).

Magnesian stones. The importance of urea-splitting bacteria in the genesis of calculi was discovered as early as 1925 (34), and is now widely recognized. The literature seems to agree that *P. ammoniae* and *P. Morgani* are always ureolytic, and that organisms of the *Micrococcus-Staphylococcus* group are frequently so. *Pseudomonas aeruginosa* (*B. pyocyaneus*) and *H. influenzae* would appear to have clinical significance, while members of the coliform and diphtheroid groups are in a doubtful position. Chute and Suby (35) find that urea-splitting organisms account for 54 per cent of all cases in the Stone Clinic of the Massachusetts General Hospital. In these cases, the hydrolysis of urea is the first of a series of chemical reactions of which the following are the most important:



The essential point in these reactions is the alkalinity caused by the free ammonia. The chief characteristic of the stones is the presence of magnesium. When found, it should always raise the question of the presence of a urea-splitting organism. The quantitative composition of the stones, however, is dependent upon the composition of the urine in which they are formed. The amount of calcium and phosphate being excreted

will obviously find reflection in the amounts of calcium carbonate and calcium phosphate found in the stone, and these may exceed the magnesium. Hellström (36) has pointed out that stones formed in association with *P. ammoniae* sometimes contain ammonium urate and calcium oxalate in addition to the salts already mentioned. We have been able to verify this observation.

The question as to whether all magnesian stones in the human are due to urea-splitting organisms or not requires further study. It is not impossible that phosphates of magnesium may be precipitated by a mechanism similar to that which may be observed in cases of phosphaturia. Such a phenomenon might take part in the formation of bladder stones. Another mechanism which might conceivably exist would be the substitution of magnesium for calcium in a calculus.

CLASSIFICATION OF A SERIES OF URINARY CALCULI

In Table I, is presented a summary of the analyses of 100 consecutive urinary calculi examined in the Medical Laboratories, McGill University Clinic, Royal Victoria Hospital. The scheme offers a new basis of classification, as it is based chiefly upon the presence or absence of the metallic elements. It has grown out of the routine use of the analytical scheme proposed in the preceding paper. Examination of the table will show that it can readily be correlated with present views of the mechanism of stone production as these have been outlined above.

TABLE I

Type	Kidney	Ureter	Bladder	Prostate urethra	Total
Uric acid	4	4	2		10
Ammonium urate			1		1
Cystine	1				1
Calcium oxalate	19	28	2	1	50
Calcium phosphate-carbonate	5	1	2	1	9
Magnesian	13	5	11		29
Total	42	38	18	2	100

In preparing the table, the stones have been arranged entirely on the basis of chemical analysis, without reference to apparent etiology or other clinical facts. It has seemed worth while to specify the anatomical site as well as the composition, as this fact has clinical significance and geo-

graphical interest, quite apart from the primary objects of this paper. Cases with stones in multiple sites have not been indicated. Recurrent stones have not been counted more than once, except in one or two instances in which a change in composition has taken place. When a change of this sort has occurred during the formation of the stone, it has been classified according to the composition of the nucleus, and the nature of the envelope has been indicated in the text.

COMMENT

Class A. Organic stones

1. Stones containing uric acid

There were 11 stones in this group. All of them have been tested by Ehrlich's diazo test to determine whether xanthine was present. All were found negative. Ten of them were composed almost entirely of uric acid. The single ammonium urate stone calls for special comment.

Case 1: A diabetic of 70 years proved to be suffering from prostatic obstruction and a large bladder calculus. Upon removal, it measured 55 mm. in diameter and weighed approximately 50 grams. The brown chalky nucleus, 30 mm. in diameter, was composed of ammonium urate, calcium carbonate, and phosphate, but was entirely free of magnesium. Surrounding it was an envelope of white chalky appearance, containing carbonate, phosphate, calcium, ammonium, and magnesium. This envelope was apparently due to the action of a urea-splitting organism. On culture, *Micrococcus*, *Aerobacter*, and a coliform organism were isolated. The nucleus would appear to have been formed in alkaline urine before the advent of the urea-splitting invader. Since the ash of the nucleus after ignition amounted to 55 per cent, it might have been classified among the carbonate-phosphate group of primary calcium stones. It seems likely that at least two mechanisms were operative in forming this nucleus.

2. Cystine stones

Only one case exhibiting cystine stones has come under observation. The patient has suffered from numerous recurrences over many years. The stones are practically pure cystine.

Class B. Primary calcium stones

This class might perhaps be said to comprise those inorganic stones which are not secondary to infection. It is important to note that stones containing carbonate and phosphate may belong here or in the magnesian group, depending on the presence or absence of magnesium. To divide the

inorganic stones in this way has etiologic significance; to call them carbonates and phosphates has none. It is worth noting that none of the stones of this class contain more than a trace of ammonia.

3. *Calcium oxalate stones*

This group, 50 in number, is by far the largest in our series, and equal to all the rest combined. It is naturally part of the class of primary calcium stones, and shows its relation to the carbonate-phosphate group by the fact that calcium carbonate and calcium phosphate are often found as minor constituents. Occasionally, they may make up the major part of the stone, and calcium oxalate, the minor one. In this way, our clinical findings tend to confirm the experimental work of Hammarsten (6, 10) which establishes the primary calcium stones as a class.

On the other hand, the oxalate stones should not be allowed to lose their identity as a group, especially for therapeutic reasons. Among 53 primary calcium stones containing oxalate, it has proved to be the major constituent in 50. It is well recognized that these stones are associated with acid urine (37), and this fact has satisfactory experimental confirmation (6, 10). Forty-six of our cases have records of urinary acidity. In 45 of them, the urine has been acid to litmus. The single case with alkaline urine had only one examination.

It is generally agreed that these stones are not caused by urinary infection (38). The clinical histories and urine studies in our cases would tend to confirm this opinion. Although significant numbers of pus cells were noted in the urine in 11 instances, these were usually scanty or absent.

One stone is of considerable interest, as it illustrates the two groups which make up the class of primary calcium stones. Another was a complication in a case of hyperparathyroidism.

Case 2. A man, 35 years of age, had suffered from recurrent calculi for 10 years. A ureteral stone weighing 339 mgm. was removed through the cystoscope. It resembled a snail shell in shape. The nucleus was composed of hard brown crystalline material, which proved to be calcium oxalate. Around it was a white chalky envelope whose major constituent was calcium phosphate; there was a minor amount of calcium carbonate and only a trace of oxalate. Magnesium was absent. It seems likely that the nucleus of this stone developed in acid

urine. Perhaps due to a change in diet, the urine deviated towards alkalinity and the salts characteristic of alkaline urine were deposited. The urine specimens examined on admission were acid to litmus. On culture, a diphtheroid organism was found, but was probably without significance, since the urine sediment contained only occasional white cells.

Case 3. A female, aged 53 years, suffered from a stone in the kidney. X-ray pictures showed an area of decalcification in the tibia which was regarded as evidence of hyperparathyroidism. The serum calcium was 12.1 mgm. per cent. The stone was removed by operation, and proved to be predominantly calcium oxalate, with a smaller amount of calcium phosphate. Exploration of the parathyroids was not carried out.

It is very interesting to observe that none of the calcium oxalate stones contained uric acid. Since both of these substances tend to precipitate in acid urine, one would expect them to occur together at times. Although such mixtures have been frequently observed in other laboratories, they seem to be very rare in the Montreal area. Fowler (39) has observed only one uric acid-calcium oxalate stone among a series of 250 examined at the Montreal General Hospital.

4. *Primary calcium phosphate-carbonate stones*

This group contains the primary calcium stones, 9 in number, in which calcium oxalate did not predominate. By analogy with animal experiments, one would expect them to have been formed in neutral or alkaline urine. In 7 of our cases in which the reaction of the urine has been recorded, it has usually been acid to litmus. These observations may, of course, reflect the hospital diets, and bear little relation to the reaction of the urine at the time the stones were being formed. Two cases of hyperparathyroidism are included in the group.

Case 4. An adult female was admitted to another institution on account of spontaneous fractures. She was bedridden and in poor physical condition. Subtotal thyroidectomy with removal of 2 parathyroids was carried out. One of the latter showed moderate enlargement. At autopsy, stones and gravel were found in the kidneys. The bones were decalcified to an extreme degree, and there was extensive metastatic calcification of the viscera. The stone specimens were composed of calcium carbonate and phosphate, without oxalate, magnesium, or uric acid.

Case 5. An adult male suffered from a series of recurrent stones over a period of 2 years. He was admitted to another hospital, where X-ray pictures of the bones showed extensive changes said to be compatible with a diagnosis of Paget's disease. The serum calcium showed values ranging from 11 to 18 mgm. per cent. Urine pH was recorded as 5.5 and 6.0. Stones were removed through the cystoscope, and at a later date by ureterotomy. They were found to consist chiefly of cal-

cium phosphate with a smaller amount of calcium carbonate. Uric acid, magnesium, and oxalate were absent.

None of the other cases in the group have shown clinical evidence pointing to excessive excretion of calcium. Among 59 cases of primary calcium stones, blood calcium values have been normal in 21, elevated in 2, and low in 1. In 35 instances, including Case 4, the determination has not been carried out.

Class C. Magnesian stones

Group 5

Twenty-nine stones in our series belong in this group. Although these stones, like group 4, contained considerable amounts of calcium carbonate and phosphate, they differed from them in containing magnesium and ammonium. The tests for these 2 substances showed a good parallelism with one another. They were present in large or moderate amounts in 27 instances, while in 2 cases they were found in small amounts. This finding is at variance with the statement, frequently made, that magnesium phosphate (presumably $Mg_3(PO_4)_2$) is one of the constituents of stones. This opinion seems to be traceable to Heller (2), who states that magnesium phosphate is to be found without ammonia, "or, at least only traces of ammonia." It seems likely that this statement has misled many subsequent writers (40, 41). I have not been able to find any quantitative data which would support such a contention.

As mentioned above, small amounts of calcium oxalate and ammonium urate have been found in some of these stones.

Cultures of the urine were made in 15 of the 29 cases. *P. ammoniae* was present 6 times, and was always associated with alkaline urine. *Micrococcus* was present 5 times, a urea-splitting *Staphylococcus* once; in 3 cases, other organisms were present. Among the 14 cases which were not cultured, there was presumptive evidence of infection in 12, while in 2 the evidence was insufficient to form an opinion. It is clear that there is a close correlation between urinary infection and the presence of magnesium as a constituent.

From this series of cases, it is impossible to say whether magnesian stones can arise in the human in the absence of urea-splitting organisms. If such cases occur at all, they are probably few in number. No magnesium carbonate stones have been observed although such have been reported in animals.

SUMMARY

1. A brief summary is given of present-day views of the chemical mechanisms underlying the formation of urinary calculi.

2. A method for classification of calculi is presented, based principally upon the presence or absence of metallic elements.

3. This method of classification has the advantage of being readily correlated with present-day views as to the chemical pathogenesis of stones.

4. A series of 100 cases is tabulated and discussed.

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BIBLIOGRAPHY

1. Wollaston, W. H., On cystic oxide, a new species of urinary calculus. *Proc. Roy. Soc., London*, 1810, 100, 223.
2. Heller, J. F., Die Harnconcretionen, ihre Entstehung, Erkennung, und Analyse mit besonderer Rücksicht auf Diagnose und Therapie der Nieren- und Blasenerkrankung. Wein, Tendler u. Comp., 1860.
3. Domanski, T. J., A classification of urinary calculi, based on composition. *Am. J. Clin. Path., Tech. Suppl.*, 1940, 4, 129.
4. Randall, A., The origin and growth of renal calculi. *Ann. Surg.*, 1937, 105, 1009.
5. Newcomb, C., The rôle of urinary colloids in the prevention of stone formation. *Indian J. M. Research*, 1930, 18, 275.
6. Hammarsten, G., Eine experimentelle Studie über Calciumoxalat als Steinbildner in den Harnwegen. *Lunds Universitets Årsskrift*, 1935-36, 32.
7. Sisk, I. R., and Toenhart, O., Factors influencing solubility of relatively insoluble salts in urine. *J. Urol.*, 1937, 37, 595.
8. Hammarsten, G., On calcium oxalate and its solubility in the presence of inorganic salts with special reference to the occurrence of oxaluria. *Compt. rend. trav. Lab. Carlsberg*, 1929, 17, No. 11. The solubility of uric acid and the primary urates in water and salt solutions at 37 deg., with special reference to the formation of sediment in the urinary passages. *Ib.*, 1931, 19, No. 7.
9. Medes, G., Solubility of calcium oxalate and uric acid in solutions of urea. *Proc. Soc. Exp. Biol. and Med.*, 1932, 30, 281.

10. Hanimarsten, G., Dietetic therapy in the formation of calcium oxalate calculi in the urinary passages. *Skand. Arch. f. Physiol.*, 1938, 80, 165.
11. Albright, F., Some medical aspects of the renal stone problem. *New England J. Med.*, 1937, 217, 1063.
12. Hammarsten, G., Harnsauresteine bei Ratten. *Skand. Arch. f. Physiol.*, 1937, 77, 33.
13. Bühler, F., Beitrag zur Frage der Urolithiasis besonders in Verbindung mit Leukaemie und Rückenmarkverletzungen. *Ztschr. f. urol. Chir.*, 1933, 37, 406.
14. Hammarsten, G., Urinary calculi and their analysis. *Nor. Med. (Hygeia)*, 1940, 7, 1329.
15. Brand, E., Block, R. J., Kassell, B., and Cahill, G. F., Cystinuria V.—The metabolism of casein and lactalbumin. *J. Biol. Chem.*, 1937, 119, 669.
16. Crowell, A. J., Cystin nephrolithiasis; Report of a case with roentgenographic demonstration of disintegration of stone by alkalization. *Surg., Gynec. and Obst.*, 1924, 38, 87.
17. Osborne, T. B., Mendel, L. B., and Ferry, E. L., The incidence of phosphatic urinary calculi in rats fed on experimental rations. *J. A. M. A.*, 1917, 69, 32.
18. McCarrison, R., The experimental production of stone in the bladder. *Indian J. M. Research*, 1926, 14, 895; 1927, 15, 197, 485, 801.
19. Higgins, C. C., Experimental production of urinary calculi. *J. Urol.*, 1933, 29, 157.
20. Dixon, W. E., and Hoyle, J. C., Effects of irradiated ergosterol in large doses. *Brit. M. J.*, 1928, 2, 832.
21. Hou, H. C., The influence of diet on the formation of urinary calculi. *Chinese M. J.*, 1936, 50, 787.
22. McCarrison, R., The influence of lime in favouring the production of stone in the bladder in rats. *Indian J. M. Research*, 1930, 17, 1101; Further researches on stone. *Ibid.*, 1930, 18, 903.
23. Van der Ryst, M. P. J., Changes in the urinary system and calculus formation in the albino rat, on a diet with a high CaCO_3 content. *Acta Brevia Neerland.*, 1936, 6, 45.
24. Ranganathan, S., Researches on "stone." *Indian J. M. Research*, 1931, 19, 1.
25. Hunter, D., and Turnbull, H. M., Hyperparathyroidism: generalized osteitis fibrosa. *Brit. J. Surg.*, 1931, 19, 203.
26. Albright, F., Baird, P. C., Cope, O., and Bloomberg, E., Studies of the physiology of the parathyroid glands. *Am. J. M. Sc.*, 1934, 187, 49.
27. Barney, J. D., and Mintz, E. R., The relation of the parathyroid glands to urinary lithiasis. *Brit. J. Urol.*, 1936, 8, 36.
28. Bauer, W., Albright, F., and Aub, J. C., A case of osteitis fibrosa cystica (Osteomalacia?) with evidence of hyperactivity of the parathyroid bodies. *J. Clin. Invest.*, 1930, 8, 229.
29. Albright, F., Aub, J. C., and Bauer, W., Hyperparathyroidism. *J. A. M. A.*, 1934, 102, 1276.
30. Barney, J. D., and Jones, G. E., Some problems in the management of urinary calculi. *J. Urol.*, 1941, 45, 1.
31. Albright, F., Sulkowitch, H. W., and Chute, R., Nonsurgical aspects of the kidney stone problem. *J. A. M. A.*, 1939, 113, 2049.
32. Higgins, C. C., and Schlumberger, F. C., Prevention of the formation of urinary calculi in patients with orthopedic problems. *Arch. Surg.*, 1937, 34, 702.
33. Flocks, R. H., Calcium and phosphorus excretion in the urine of patients with renal or ureteral calculi. *J. A. M. A.*, 1939, 113, 1466. Prophylaxis and medical management of urinary lithiasis: the role of the quantity and precipitability of the urinary calcium. *J. Urol.*, 1940, 44, 183.
34. Hager, B. H., and Magath, T. B., The etiology of encrusted cystitis with alkaline urine. *J. A. M. A.*, 1925, 85, 1352.
35. Chute, R., and Suby, H. I., Prevalence and importance of urea splitting bacterial infections of the urinary tract in the formation of calculi. *J. Urol.*, 1940, 44, 590.
36. Hellström, J., Staphylococcus Stones. A Clinical Study of 90 Cases. *Norstedt and Sons, Stockholm*, 1936.
37. Burkland, C. E., Etiology and prevention of oxalate calculi in urinary tract: plan of therapy. *J. Urol.*, 1941, 46, 82.
38. Higgins, C. C., Recurrent renal lithiasis. *J. Urol.*, 1938, 40, 184.
39. Fowler, A. F., Personal communication.
40. Domanski, T. J., Analysis of urinary calculi. *Am. J. Clin. Path., Tech. Suppl.*, 1938, 2, 157.
41. Bodansky, M., and Bodansky, O., *The Biochemistry of Disease*, The MacMillan Co., New York, 1940.

THE RELATION OF HIPPURIC ACID EXCRETION TO THE VOLUME OF THE URINE^{1,2}

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The data submitted in this presentation indicate that the amount of benzoic acid excreted in the urine as hippuric acid in the Quick intravenous test (1) for liver function is influenced to a considerable extent by the volume of the simultaneous output of urine. This factor may account for some of the high values that at times have been observed in normal individuals, and even in patients with hepatic disease. The data also show a lack of correlation between body weight, bromsulphalein excretion, and the results of the Quick test.

Before Quick (2) introduced the hippuric acid test for liver function, it had been employed by Kingsbury and Swanson (3) and by Bryan (4) as a measure of renal function. Thus, for the proper interpretation of the test in liver disease, it would seem that adequate renal function should be present, because an impairment of the latter would depress the rate of excretion of the hippuric acid and so mimic hepatic dysfunction. Accordingly some workers have regarded, as a prerequisite for the proper interpretation of the test in hepatic disease, normal renal function, as judged by the urea clearance test or by the absence of a retention of blood urea nitrogen. Quick (5) believed, however, that the rate of hippuric acid excretion by the kidneys is 50 per cent higher than its rate of synthesis in the liver and that, as a result, only very serious disorders of renal function would affect its elimination in the urine. Thus it has been alleged that a normal excretion of hippuric acid can be obtained despite a greatly diminished urea clearance or an increased urea content of the blood (6, 7).

The effect of the volume of the output of the urine on the amount of hippuric acid eliminated

has received little attention, though Snapper and Grünbaum (8) noted that nephritic patients, having an abnormal retention of urea, may eliminate more hippuric acid in a large than in a small output of urine. Probststein and Londe (7), on the other hand, found no relation between urinary volume and the output of hippuric acid in 14 normal subjects.

A small excretion of hippuric acid, together with a small volume of urine, was observed in certain subjects in whom all other tests of hepatic function were negative. At first, this was thought to be due to incomplete evacuation of the bladder, but a similar result was obtained when we resorted to catheterization. Then, a very high value for hippuric acid excretion was observed in a patient who had a decompensated portal cirrhosis. He passed at the same time a very large volume of urine. He had a blood urea nitrogen concentration of 5 mgm. per cent, and it seemed probable that, in him, a simple diuresis was occurring. On the basis of these observations, we decided to determine whether or not any significant correlation could be established between the amount of the hippuric acid excreted and the simultaneous volume of the urine in our series of cases in which hippuric acid tests were being done.

Because Mateer (9) suggested that the size of the individual might influence the results of the test, we also examined for a correlation between body weight and the amount of hippuric acid excreted in the urine. We have included, in addition, data on the results of the bromsulphalein test because a difference of opinion (9 to 12) exists in the literature concerning the relative sensitivity of the bromsulphalein and the hippuric acid tests.

DESCRIPTION OF SUBJECTS

The hippuric acid test was performed on 109 patients in the medical and surgical wards or in the gastro-

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² Aided by a grant from the Smith, Kline and French Laboratories.

TABLE I

Data on 100 cases of evident or suspected hepatic disease

The high values for benzoic acid excretion in cases 1 and 2 were checked by duplicate analyses but cannot be explained at this time.

Case number	Sex	Body weight	Serum bilirubin	Icteric index	Blood urea nitrogen	Dye retention	Benzoic acid*	Volume of urine	Clinical diagnosis
		lbs.	mgm. per 100 cc.	units	mgm. per 100 cc.	per cent	grams	cc.	
1.	M	142		6	4	12	1.70	315	Laennec's cirrhosis.
2.	M	124		7	15	0	1.60	140	Cystinuria.
3.	M	121	0.5		8	8	1.44	164	Hodgkin's disease.
4.	M	139	±0.1		10	0	1.17	136	Idiopathic hypoglycemia.
5.	F	156		9	10	4	1.17	84	Chronic passive hepatic congestion, hypertensive cardiovascular disease.
6.	F	120		<10	5	0	1.12	425	Chronic calculeous cholecystitis, diabetes mellitus.
7.	F	95		7	10	0	1.11	126	Diabetes mellitus.
8.	F	124	±0.1		8	0	1.10	160	Arsphenamine exfoliative dermatitis.
9.	F	130	±0.1		11	0	1.10	136	Toxic diffuse goitre.
10.	M	178	±0.1		17	34	1.10	116	Laennec's cirrhosis, gout.
11.	M	184		7	9	12	1.08	160	Toxic diffuse goitre.
12.	M	198		6	14	8	1.04	98	Idiopathic hepatosplenomegaly.
13.	M	117		6	13	40	1.00	212	Chronic atrophic gastritis.
14.	M	156		10	7	8	1.00	84	Chronic cholangitis.
15.	F	156		6	8	0	0.99	120	Chronic myelogenous leukemia.
16.	F	55	±0.1		11	0	0.96	100	Hepatolenticular degeneration.
17.	F	112	±0.1		9	0	0.95	130	Toxic diffuse goitre.
18.	F	147	0.4		14	4	0.95	60	Partial stenosis of common bile duct.
19.	M	175		10	12	0	0.95	222	Chronic alcoholism.
20.	F	85	±0.1		8	0	0.93	318	Toxic diffuse goitre.
21.	M	123		8	2	4	0.91	112	Toxic diffuse goitre.
22.	F	106		5	8	24	0.91	100	Aleukemic leukemia.
23.	F	112	±0.1		8	8	0.91	100	Weber-Christian syndrome, hypertensive cardiovascular disease.
24.	M	174	±0.1		12	9	0.90	258	Toxic diffuse goitre.
25.	F	154	±0.1		8	0	0.89	195	Idiopathic hepatomegaly.
26.	M	122		8	7	0	0.87	100	Idiopathic hepatomegaly, psoriasis.
27.	M	114	±0.1		11	8	0.87	342	Diffuse toxic goitre, diabetes mellitus.
28.	F	134		6	7	12	0.84	190	Diffuse toxic goitre.
29.	F	153	±0.1		8	0	0.84	164	Hodgkin's disease.
30.	M	156		10	8	20	0.83	50	Chronic calculeous cholecystitis.
31.	F	136		8	11	0	0.82	110	Idiopathic hepatomegaly, achlorhydria.
32.	M	145		6	17	20	0.82	54	Secondary hepatic carcinoma.
33.	M	141	±0.1		6	32	0.80	224	Idiopathic hepatosplenomegaly, diabetes mellitus.
34.	F	144		14	12	12	0.80	178	Banti's syndrome.
35.	F	144		7	9	16	0.80	74	Hodgkin's disease.
36.	F	110		10	11	12	0.80	90	Idiopathic hepatomegaly, right hydronephrosis.
37.	F	108		10	8	4	0.79	48	Toxic diffuse goitre.
38.	F	97		6	8	0	0.77	88	Toxic nodular goitre, hypertensive cardiovascular disease.
39.	F	130	±0.1		7	0	0.77	82	Post-splenectomy, esophageal varices with hemorrhage.
40.	F	137	±0.1		11	0	0.77	56	Acute disseminated lupus erythematosus.
41.	M	185		7	11	40	0.74	185	Chronic passive hepatic congestion.
42.	M	158		4	12	48	0.71	296	Idiopathic hepatomegaly.
43.	M	142		7	8	26	0.70	53	Laennec's cirrhosis.
44.	M	121	±0.1		11	16	0.70	108	Toxic nodular goitre, auricular fibrillation.
45.	F	106		6	15	24	0.70	68	Toxic nodular goitre, hypertensive cardiovascular disease.
46.	F	125		6	11	36	0.70	40	Idiopathic hepatomegaly, hypoglycemia.
47.	M	119		10	12	12	0.70	40	Pyrexia of unknown origin.
48.	F	129	0.7		13	16	0.70	43	Secondary anemia.
49.	M	114		12	13	24	0.70	128	Toxic diffuse goitre.
50.	F	190		7	12	12	0.70	76	Diabetes mellitus, hepatomegaly.
51.	F	101		6	11	10	0.70	130	Laennec's cirrhosis.
52.	M	145		11	12	48	0.70	65	Laennec's cirrhosis, hypertensive cardiovascular disease.
53.	M	132		7	10	0	0.70	161	Hepatomegaly, diabetes mellitus.
54.	M	222	0.4		11	28	0.69	100	Decompensated Laennec's cirrhosis.

* Excretion in urine in one hour after intravenous injection of 1.77 grams.

TABLE I—Continued

Case number	Sex	Body weight	Serum bilirubin	Icteric index	Blood urea nitrogen	Dye retention	Benzoic acid	Volume of urine	Clinical diagnosis
		lbs.	mgm. per 100 cc.	units	mgm. per 100 cc.	per cent	grams	cc.	
55.	F	103		12	8	12	0.68	126	Chronic calculous cholecystitis, diabetes mellitus.
56.	F	142	±0.1		7	0	0.68	48	Hepatomegaly, exfoliative dermatitis.
57.	F	123	±0.1		9	12	0.68	100	Toxic diffuse goitre.
58.	F	146	±0.1		13	16	0.68	96	Toxic nodular goitre, chronic passive hepatic congestion.
59.	M	183		7	8	20	0.68	60	Congenital hypoplasia of liver.
60.	F	136		6	9	0	0.68	95	Idiopathic hypoglycemia.
61.	M	144		4	17	16	0.68	80	Chronic calculous cholecystitis, secondary anemia.
62.	F	220	±0.1		12	20	0.66	48	Chronic calculous cholecystitis, diabetes mellitus.
63.	M	195		12	11	8	0.64	52	Polycythemia vera.
64.	F	160	±0.1		8	8	0.63	80	Toxic nodular goitre, auricular fibrillation, chronic hepatic passive congestion.
65.	M	104		3	8	0	0.60	45	Post-splenectomy, Banti's syndrome.
66.	F	144		14	12	12	0.60	80	Banti's syndrome.
67.	F	134	±0.1		14	48	0.60	84	Chronic calculous cholecystitis, renal calculus, pyelonephritis.
68.	M	141		5	22	32	0.60	174	Laennec's cirrhosis.
69.	F	106	0.3		10	16	0.60	96	Chronic lymphocytic leukemia.
70.	M	151	0.4		8	16	0.60	80	Chronic lymphocytic leukemia.
71.	M	182	0.4			0	0.59	55	Idiopathic hepatomegaly.
72.	M	137		14	15	36	0.56	200	Polyserositis.
73.	F	130	±0.1		16	28	0.55	85	Toxic nodular goitre, auricular fibrillation, chronic hepatic passive congestion.
74.	F	103		8	8	8	0.54	84	Subacute bacterial endocarditis.
75.	F	131	±0.1		8	8	0.54	84	Chronic hepatic passive congestion, hypertensive cardiovascular disease.
76.	F	148		7	8	36	0.54	43	Chronic lymphocytic leukemia.
77.	F	160	±0.1		10	48	0.54	70	Toxic nodular goitre, auricular fibrillation, chronic hepatic passive congestion.
78.	M	132	±0.1		19	12	0.50	140	Laennec's cirrhosis.
79.	F	124		6	10	0	0.50	110	Hodgkin's disease.
80.	F	142		8	15	15	0.50	64	Laennec's cirrhosis.
81.	M	173		9	16	36	0.50	90	Chronic passive hepatic congestion, hypertensive cardiovascular disease.
82.	M	182		9	19	24	0.50	202	Chronic passive hepatic congestion, hypertensive cardiovascular disease.
83.	F	90		6	9	0	0.50	200	Toxic diffuse goitre.
84.	F	115	±0.1		17	14	0.48	34	Chronic passive hepatic congestion, luetic cardiovascular disease.
85.	F	111	±0.1		16	16	0.48	156	Primary anemia, diabetes mellitus, toxic nodular goitre.
86.	F	86		4	16	0	0.44	90	Scurvy.
87.	F	125	±0.1		9	0	0.41	100	Xanthomatosis.
88.	F	100		7	13	5	0.40	86	Diffuse toxic goitre.
89.	F	155	±0.1		14	16	0.40	36	Diffuse toxic goitre, hypertensive cardiovascular disease.
90.	F	121		6	14	24	0.36	226	Primary anemia.
91.	F	105		7	12	12	0.33	50	Idiopathic hepatomegaly, scurvy.
92.	M	145		11	12	48	0.32	65	Laennec's cirrhosis, hypertensive cardiovascular disease.
93.	F	135		5	13	20	0.32	75	Multiple cystic disease of the liver.
94.	F	130	±0.1		15	32	0.30	34	Diffuse toxic goitre, chronic passive hepatic congestion.
95.	F	139		9	11	36	0.24	48	Congenital cystic disease of the liver.
96.	M	110	±0.1		21	8	0.25	80	Toxic nodular goitre, hypertensive cardiovascular disease.
97.	F	95		11	10	0	0.22	84	Chronic passive hepatic congestion, malignant hypertension.
98.	F	85		4	9	4	0.18	42	Chronic passive hepatic congestion, hypertensive cardiovascular disease, toxic nodular goitre.
99.	F	243		7	8	32	0.14	15	Chronic pancreatitis, diabetes mellitus.
100.	F	102		9	9	36	0.11	20	Chronic passive hepatic congestion, toxic nodular goitre, hypertensive cardiovascular disease.

intestinal clinic of the University of Pennsylvania Hospital. In all of them, hepatic disease was manifest or suspected, either because the liver was significantly enlarged or because the patient had a disease in which hepatic dysfunction may occur (Table I). In each case, the blood urea nitrogen concentration was normal and the direct van den Bergh reaction, negative. The body weight, serum bilirubin or icteric index, and the presence or absence of retention of bromsulphalein in the blood serum one half hour after its injection were determined in all instances.

In 12 other subjects (all females), a comparative study of the hippuric acid test, performed in the usual fashion, and after giving an excess of water to increase urine

volume, was made (Figure 1). Five of these (Cases 1, 2, 3, 4, 5) were apparently normal individuals and had a negative direct van den Bergh reaction, a normal serum bilirubin level, no retention of bromsulphalein in the blood serum after one half hour, and a negative hippuric acid test. One (Case 6), with chronic glomerulonephritis, had an elevated blood urea nitrogen value (62 mgm. per cent), a urea clearance of 20 per cent and negative van den Bergh and bromsulphalein tests. Three patients (Cases 7, 8, and 9) had some retention of bromsulphalein (4, 10 and 16 per cent), their direct van den Bergh reactions being negative or delayed, and their diagnoses being respectively non-toxic nodular goitre, diabetes mellitus with peripheral neuritis, and diffuse toxic goitre.

SODIUM BENZOATE

1.4 GM.

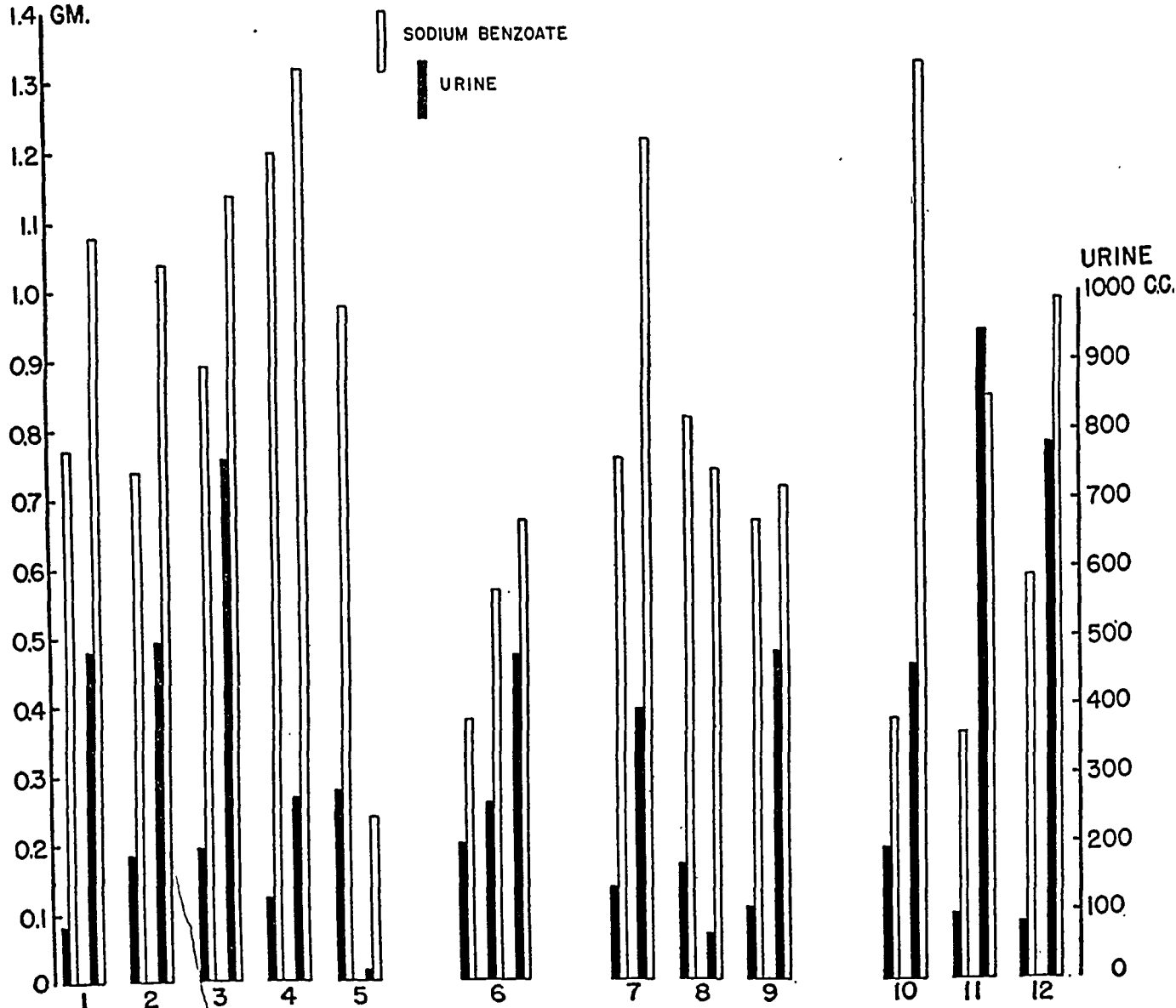


FIG. 1. COMPARISON OF RESULTS OF THE HIPPURIC ACID TEST WHEN PERFORMED WITHOUT CONTROL OF THE FLUID INTAKE AND WHEN THE FLUID INTAKE WAS PURPOSELY INCREASED

The first pair of columns in each case represents the volume of urine and the amount of hippuric acid, in terms of benzoic acid, excreted during the test when performed under the usual conditions; the second pair, the results when the fluid intake was increased.

Erratum: Measurements are of benzoic acid, not sodium benzoate, in this and the following figures.

Case 10, an acromegalic, manifested a biphasic direct van den Bergh reaction, a slight elevation of the serum bilirubin (0.6 mgm. per cent), and a low value for hippuric acid excretion. The remaining 2 patients (Cases 11 and 12) were deeply jaundiced, had immediate direct van den Bergh reactions, very high icteric indices (66 and 112), and low values for hippuric acid excretion. The diagnoses in these 2 patients were biliary cirrhosis (Case 11) and acute catarrhal jaundice (Case 12).

METHODS

The intravenous 1 hour hippuric acid test was performed by the method of Quick (1) with modifications proposed by Weichselbaum and Probststein (13). The amount of hippuric acid in the urine was determined by titration with sodium hydroxide. One hour after a breakfast of 1 cup of coffee and 2 slices of toast, the patient was instructed to empty the bladder completely. Immediately thereafter, 20 cc. of an 8.85 per cent solution of sodium benzoate (1.77 grams) were injected intravenously, the duration of time for the injection being 5 minutes. One glass of water (180 cc.) was then ingested. One hour following the completion of the injection of the benzoate solution, the bladder was emptied by catheterization in all females (62) and in all those males (6) who could not assume the erect posture to void. The remaining 32 males emptied their bladders by voiding and had no evidence of prostatism.

The bromsulphalein test was performed either on the day before or on the day after the hippuric acid test. The amount of dye used was 5 mgm. per kilogram of body weight, a single sample of venous blood being removed from the opposite arm 30 minutes after injection of the dye. The presence of any dye in the serum at the end of this time was considered as indicative of impaired excretory ability of the liver (14). The patients were required to be in the fasting state on the morning of the test. The icteric index, blood urea nitrogen, and serum bilirubin determinations were made by accepted standard routine laboratory procedures.

In those patients in whom we wished to determine the effect of water diuresis on the excretion of the hippuric acid, 360 cc. of water were administered $1\frac{1}{2}$ and $\frac{1}{2}$ hours before the injection of the sodium benzoate, as well as immediately afterward. Thus, these patients received a total of 1080 cc. of water in addition to the fluid in the cup of coffee and the 20 cc. of sodium benzoate solution injected intravenously. The bladders in all of these patients were completely emptied by catheterization 1 hour after the end of the injection of the sodium benzoate solution.

RESULTS

A. Relation of hippuric acid excretion to body weight

No significant correlation between the amount of benzoic acid excreted as hippuric acid and the

body weight of the subjects was found (Figure 2). The correlation coefficient (r) was ± 0.074 .

B. Relation of hippuric acid excretion to the volume of urine

In the 100 cases included in Table I, a direct correlation existed between the amount of hippuric acid eliminated through the kidneys and the volume of the urine secreted in the same period. When the range of hippuric acid excreted, in terms of benzoic acid, was divided into 5 groups in the order of increasing values, the average volume of the urine in the particular groups increased directly (Table II). This relationship was found to be mathematically significant when the values were plotted against urinary volume in all of the tests (194) performed in the clinic to date (Figure 3). The subjects included normals as well as persons with evident or suspected hepatic disease. The correlation coefficient (r) was ± 0.480 .

C. Effect of increasing the urinary volume on the amount of hippuric acid excreted

The urinary output and, at the same time, the amount of excreted hippuric acid were increased in 10 of the 12 patients in whom on a second test the fluid intake was increased (Figure 1). In 4 of the 5 control subjects (Cases 1, 2, 3, 4), the volume of urine was increased from an average of 146 to 501 cc., while the average benzoic acid excretion was increased from 0.9 to 1.14 grams. In 2 of the subjects, 1 a control (Case 5) and 1 with mild hepatic damage (Case 8), there was a decrease in the volume of urine as well as in the amount of benzoic acid. Both of these subjects complained of intense nausea during and after the period when large amounts of water were being ingested. The 3 patients with bilirubinemia and dye retention (Cases 10, 11 and 12) had subnormal values for benzoic acid excretion when the test was performed by the standard technique. In all of them, the benzoic acid excretion was increased to normal or above by inducing a water diuresis. In Case 6, with a urea nitrogen retention of 62, the benzoic acid excretion increased from 0.38 to 0.57 and then to 0.67 gram as the urine volume increased from 200 to 260 and finally to 475 cc. (Figure 1).

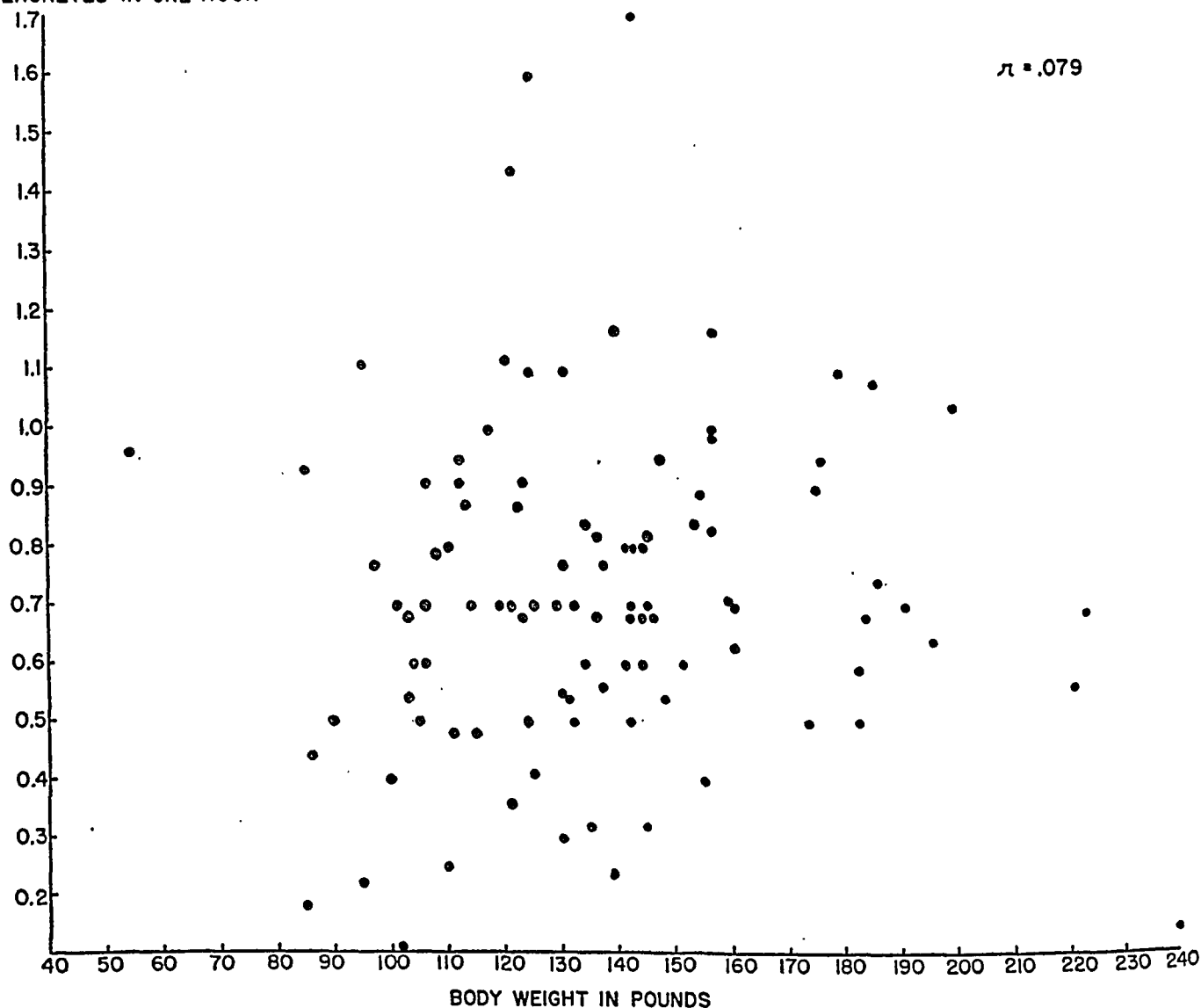
GRAMS SODIUM BENZOATE
EXCRETED IN ONE HOUR

FIG. 2. THE RELATION OF BODY WEIGHT TO THE AMOUNT OF HIPPURIC ACID EXCRETED, IN TERMS OF BENZOIC ACID

Correlation coefficient, (r) 0.079, is not significant.

D. Relation of hippuric acid excretion to the presence or absence of bromsulphalein retention

In the 100 cases with a negative direct van den Bergh reaction, the excretion of hippuric acid was below normal in 47, while bromsulphalein retention was present in 72. Both tests were positive in 38 cases and negative in 19. The dye test was positive and the hippuric acid test negative in 34, while the reverse was true in 9 (Table III). In 16 of the cases, the benzoic acid excretion was greater than the upper normal of 0.95 gram, yet in 8 of these, dye retention was present (Table I). In some series of cases studied by

other authors (9), the hippuric acid test has been observed to be more sensitive than the bromsulphalein test.

COMMENT

The data herein presented indicate that, irrespective of the ability of the body to synthesize hippuric acid, its elimination through the kidneys is influenced by the volume of the urine secreted during the test period. The ability to influence the amount of hippuric acid excreted in individual subjects by varying the volume of their urine, incident to the administration of fluid, strongly supports the correlation in the data on single

tests which we have presented. This relationship between the volume of the urine and the amount of hippuric acid excreted presents some objection to the use of hippuric acid elimination as a test for hepatic function, unless correction can be made for variations in urinary volume.

Possibly this relationship may account for some of the high values for the test observed by Mateer (9) in some of his controls, and for the higher than normal values observed by Rosenberg (15) in some patients with hepatic disease, and attributed by him to a hyperirritable phase of damage.

The low values for the 2 subjects (Cases 5, 8) in whom we failed to induce a water diuresis deserve brief comment. The subjects drank the

fluid, and none of the urine was lost. Both of them were extremely nauseated during the test. The low urine volume outputs could perhaps be explained on the basis of failure of the fluid to leave the stomach and so reach the intestine for absorption because of duodenal spasm, which, according to Ingelfinger (16), may occur during nausea.

The attempt to find the one most sensitive test of impaired hepatic function has led to conflicting results and views. In some series of cases (9) impaired hippuric acid excretion has been more frequent than retention of bromsulphalein. In our series, the reverse was the case. After making due allowance for such factors as the influ-

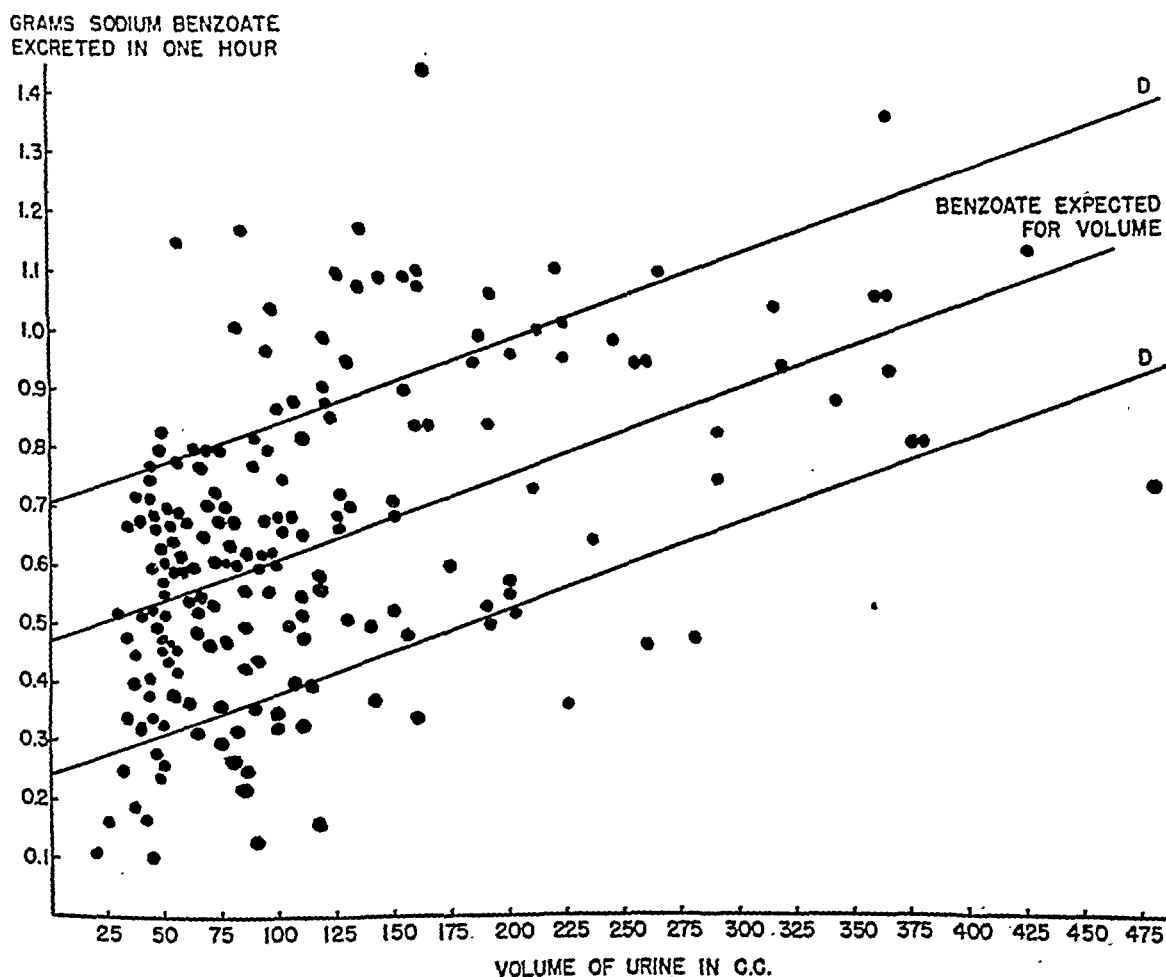


FIG. 3. THE RELATION OF VOLUME OF URINE TO THE AMOUNT OF HIPPURIC ACID, EXCRETED AS BENZOIC ACID, IN 194 CASES OF EVIDENT OR SUSPECTED HEPATIC DISEASE

The correlation coefficient (r) $+0.480$, is significant.

D indicates standard deviation.

TABLE II

The relation of the average volume of urine eliminated during the test period to the amount of hippuric acid excreted, in terms of benzoic acid

Amount of benzoic acid excreted	Average volume of urine	Number of cases
grams	cc.	
0-0.29	48 (15-84)	6
0.3-0.49	86 (34-226)	11
0.5-0.69	99 (45-202)	30
0.7-0.95	125 (40-342)	37
>0.95	161 (84-425)	16

ence of urine volume output, impairment of renal excretion, and variation of technique, there still remain differences from case to case as to which test first exhibits impairment. The use of several tests of hepatic function will, therefore, reveal an early disturbance of function more often than will any single test.

TABLE III

Summary of results on 100 hippuric acid and bromsulphalein tests

Impaired hippuric acid synthesis	Retention of dye	Number of cases
+	+	38
+	0	9
0	+	34
0	0	19

The dye test is almost always positive when an impairment of hepatic excretory ability is already indicated by an immediate or biphasic direct van den Bergh reaction. It is for this reason that we required that the direct van den Bergh test be negative in each of the 100 cases in which the results of the dye and the hippuric acid test were to be compared.

SUMMARY

In a series of 100 patients with manifest or suspected hepatic disease, a significant direct correlation was found between the amount of hippuric acid eliminated by the kidneys and the volume of the urine secreted during the same period. A higher than normal value for benzoic acid excretion was associated with a large volume of

urine. A low value in some patients with hepatic disease could be increased to normal or above by inducing a water diuresis. A low value for benzoic acid excretion in one patient with excessive blood urea nitrogen retention was raised almost to normal by a similar procedure. The dependence of the hippuric acid test on the rate of that substance's excretion by the kidneys constitutes some objection to its use as an index of the detoxifying ability of the liver.

Bromsulphalein retention occurred in a higher percentage of our cases than did an impaired hippuric acid excretion.

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BIBLIOGRAPHY

1. Quick, A. J., Ottenstein, H. N., and Weltchek, H., Synthesis of hippuric acid in man following intravenous injection of sodium benzoate. *Proc. Soc. Exper. Biol. and Med.*, 1938, 38, 77.
2. Quick, A. J., The synthesis of hippuric acid: A new test of liver function. *Am. J. M. Sc.*, 1933, 185, 630.
3. Kingsbury, F. B., and Swanson, W. W., Synthesis and elimination of hippuric acid in nephritis: A new renal function test. *Arch. Int. Med.*, 1921, 28, 220.
4. Bryan, A. W., Clinical and experimental studies on sodium benzoate: Value of sodium benzoate test of renal function, and the effect of injury of liver on hippuric acid synthesis. *J. Clin. Invest.*, 1925, 2, 1.
5. Quick, A. J., Clinical value of the test for hippuric acid in cases of disease of the liver. *Arch. Int. Med.*, 1936, 57, 544.
6. Fouts, P. J., Helmer, O. M., and Zerfas, L. G., The secretion of hippuric acid in pernicious anemia. *Am. J. M. Sc.*, 1937, 193, 647.
7. Probst, J. G., and Londe, S., Studies of liver function by means of Quick's hippuric acid test. *Ann. Surg.*, 1940, 111, 230.
8. Snapper, J., and Grünbaum, A., Der hippursäurestoffwechsel bei nierkrankheiten. *Klin. Wchnschr.*, 1924, 3, 101.
9. Mateer, J. G., Baltz, J. I., Marion, D. F., and Hollands, R. A., A comparative evaluation of the newer liver function tests. *Am. J. Digest. Dis.*, 1942, 9, 1.
10. Boyce, F. F., and McFetridge, E. M., Studies of hepatic function by the Quick hippuric acid test.

- III. Various surgical states. *Arch. Surg.*, 1938, 37, 443.
11. De Lor, C. J., and Reinhart, H. L., Analysis of hippuric acid, galactose tolerance, bromsulphthalein and prothrombin tests in 381 cases. *Am. J. Clin. Path.*, 1940, 10, 617.
12. White, F. W., Deutsch, E., and Maddock, S., The comparison of serial hippuric acid excretion, total cholesterol, cholesterol ester and phospho-lipid tests in diseases of the liver. A clinical comparison of the tests. *Am. J. Digest. Dis.*, 1940, 7, 3.
13. Weichselbaum, T. E., and Probst, J. G., Determination of hippuric acid in urine. *J. Lab. and Clin. Med.*, 1939, 24, 636.
14. Helm, J. D., and Machella, T. E., The significance of dosage and time factors on the value of the bromsulphthalein test for liver function. *Am. J. Digest. Dis.*, 1942, 9, 141.
15. Rosenberg, D. I., Discussion of paper by Mateer (9).
16. Ingelfinger, F. J., and Moss, R. E., The activity of the descending duodenum in man during nausea produced by caloric stimulation of the semi-circular canals. *Proc. Am. Physiol. Soc.*, 1942, 1, 43.
17. Miller, T. G., and Machella, T. E., Diseases of the liver. *Nelson Loose Leaf Med.*, Thomas Nelson and Sons, New York, 1941, 5, 479.

SERUM IODINE FRACTIONS IN HYPERTHYROIDISM^{1,2}

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In untreated hyperthyroid patients, blood or serum iodine has been found to be above the normal range (1). Whether this iodine occurs in a protein-like or an inorganic form has been investigated by a number of workers, employing a variety of methods (2). Previously reported experiments from this laboratory indicated that most of the iodine was contained in the serum in the organic or protein-like form (3). It was only after iodine salts had been ingested that readily demonstrable amounts of inorganic iodine were found in the blood serum of either euthyroid or hyperthyroid people. The present investigation deals with the behavior of the serum protein-bound iodine, during iodine treatment of 15 hyperthyroid patients.

In comparison with other methods, Somogyi's zinc sulfate precipitation (4) has proved a simple and effective technique for the separation of the iodine fractions, in 6 cc. aliquots of serum of patients receiving inorganic iodine. A study has been made of the effect on the serum protein-bound iodine of giving Lugol's solution to euthyroid subjects. The kinds of iodine containing compounds precipitated by zinc sulfate and sodium hydroxide have also been investigated.

REVIEW OF METHODS

Trevorrow (5), Alpert (6), and Lein (7) have all used Somogyi's zinc sulfate precipitation method for the separation of iodine fractions, but their investigations have not dealt with protein-bound iodine in serum of hyper- or hypothyroid patients. Trevorrow worked with blood after the *in vitro* addition of thyroxine or potassium iodide (5). Alpert has used the filtrate from precipi-

tated plasma for the estimation of diodrast iodine (6). Lein has used filtrate for evaluation of ionic iodide after the intravenous injection of potassium iodide solution in iodide tolerances (7).

The zinc sulfate precipitation method was selected because previously described methods for differentiating iodine fractions, such as precipitation with organic solvents, dialysis and precipitation of proteins with heat and acetic acid, were either inaccurate or time consuming. Trevorrow, after repeated and prolonged extraction of blood with ethyl alcohol, was able to remove completely all of the iodine from the insoluble protein fraction (with ethanol "30 to 50 per cent being removed in the first fraction, 30 to 45 per cent in the four hour continuous extraction and the remainder in the next 24 hours") (5). Boyd has recently confirmed these observations, that in blood the amount of iodine dissolved by alcohol depends on the efficiency of extraction (8). Trevorrow also found that after 4 acetone extractions, each 1 to 12 hours in length, all of the blood iodine was soluble in acetone (5). On the other hand Davison, Zollinger, and Curtis, who did not make such prolonged extractions with acetone, have tried to use acetone to fractionate the iodine in blood (9, 10). McClendon and Foster (11) employed methanol precipitation of blood iodine. It is probable, however, that with prolonged extractions methanol might be found to dissolve as much blood iodine as the ethanol and acetone employed by Trevorrow. From her work, it has been concluded that organic solvents do not separate potassium iodide or thyroxine from the iodine normally occurring in blood.

Bassett, Coons, and Salter precipitated proteins with heat and acetic acid to separate inorganic from "protein-bound" iodine. However, when the serum inorganic iodine was elevated, as after the administration of iodine to hyperthyroid subjects, there was a "spurious elevation" of protein iodine. They thought that this elevation might "be due to occlusion or adsorption and thus

¹ This investigation was aided by a grant from the Knight Fund and Fluid Research Funds of the Yale University School of Medicine.

² Presented in part before the Federation of American Societies for Experimental Biology, Boston, Massachusetts, April, 1942.

might be regarded as a chemical artefact" (12, 13).

Riggs, Laviates and Man (3) dialyzed blood serum and were able to separate potassium iodide from serum iodine. However, this method was time consuming and would not be practicable for clinical purposes.

PRECIPITATION METHOD

Serum is precipitated with the reagents used by Somogyi (4) to prepare filtrate for blood sugar determinations. The precipitate is washed with distilled water. Iodine in precipitate is determined by the Riggs and Man permanganate acid ashing method (14).

Reagents

Solution I—"12.5 grams of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ are dissolved in water, 125 cc. of 0.25 N H_2SO_4 are added, and the mixture is diluted with water to 1 liter."

Solution II—0.75 N sodium hydroxide. "The two solutions must be so related that when 50 cc. of Solution I are titrated with Solution II, 6.70 to 6.80 cc. are required to produce a permanent pink color with phenolphthalein. The titration must be carried out by slow addition of the sodium hydroxide with continuous shaking" (4).

Procedure

One volume of serum, 8 volumes of zinc sulfate solution, and 1 volume of sodium hydroxide are shaken thoroughly. For usual sera, 6 cc. of serum, 48 cc. of zinc sulfate solution, and 6 cc. of sodium hydroxide are convenient aliquots. The mixture is then filtered through 11 cm. Whatman number 42 filter paper. The precipitate is washed 8 to 12 times with iodine-free distilled water. Stirring the precipitate with a blunt stirring rod facilitates and accelerates the washing. (The washings may be tested with dilute silver nitrate solution acidified with nitric acid. A white precipitate indicates that some chloride is still mixed with the protein precipitate. As serum contains much more chloride than inorganic iodide, the latter substance is undoubtedly completely removed when silver chloride precipitate does not form.)

The iodine determination is made by the Riggs and Man permanganate acid ashing method (14). This method has recently been shortened because only 1 heating was found necessary for complete digestion of the organic iodine compounds in serum.

The precipitate and filter paper are transferred to a 1200 cc. iodine distilling flask and the iodine determination is continued as if serum had been used. If 6 cc. of serum were precipitated, 15 grams of potassium permanganate and 210 cc. of 18 N sulfuric acid are used to digest the filter paper and precipitate. When the flasks are heated to about 100°C ., excessive foaming may occur. Therefore, the flames should be low until the mixture begins to boil. If foaming is excessive, heating should be stopped and distilled water may be added to

the contents of the flask. However, if the first 80 cc. of 18 N sulfuric acid are added in 2 portions so that a moderate reaction occurs, subsequent application of heat does not cause excessive foaming. It is on account of this foaming that a 1200 cc. rather than a 900 cc. iodine distilling flask should be used. An oscillating machine which shakes all the flasks back and forth during digestion has proved efficient in preventing excessive bumping during digestion.

After heating the flask and contents to 195 degrees centigrade, 80 cc. of distilled water are used to dilute the mixture before distillation.

Iodine in the filtrate may also be determined. The aliquot used depends on the probable inorganic iodine content of the serum. If the patient has been taking iodides, 10 to 20 cc. of filtrate should be sufficient. If he has had no inorganic iodine, as much filtrate as possible (40 to 45 cc.) should be used.

Protein bound iodine in serum of euthyroid subjects given Lugol's solution

In Table I are the data on 6 euthyroid subjects who were given 10 to 45 drops of Lugol's solution per day during lengths of time ranging from 1 to 7 days. The table gives the dosage of Lugol's and the number of days that elapsed between the time that administration was discontinued and the final determination of total iodine. Duplicate and average values for the total and precipitable iodines are given in order to show the agreement between the duplicates of precipitable iodine.

During Lugol's administration, the total iodines were enormously increased, ranging from 29 to 522 gamma per cent. In the first experiment, the serum total iodines before and several days after omission of Lugol's were 5.1 gamma per cent; the precipitable iodines were 5.6 and 5.9 when the patient had taken sufficient Lugol's to elevate the total iodine to 50 and 29 gamma per cent. In the second experiment, there was just as satisfactory agreement between the total iodines of 5.0 and 5.5 gamma per cent before and after Lugol's, and precipitable iodines of 5.7 and 5.9 gamma per cent when total iodines were above 100 gamma per cent. In the last 4 experiments, with the exception of one aliquot of precipitable iodine of patient 2831, the serum total iodines after Lugol's agreed within 1.6 gamma per cent with the serum precipitable iodines during Lugol's administration. That the precipitable iodine of a euthyroid individual on Lugol's solution is the same as the total iodine when not taking Lugol's

TABLE I

Serum iodines of patients with normal thyroids who were given Lugol's solution

Patient number	Date	Lugol's			Serum iodine gamma per cent					
		Drops daily	Days given	Days since Lugol's	Total			Precipitable		
					Duplicates		Average	Duplicates		Average
2782	October 14, 1941		0		4.9	5.3	5.1			
	October 16, 1941	30	2		49.0	50.6	49.8	5.8	5.3	5.6
	October 21, 1941	10	7		29.2	28.8	29.0	5.4	6.3	5.9
	October 24, 1941			2	4.9	5.4	5.1			
2798	October 22, 1941		0		4.6	5.4	5.0			
	October 27, 1941	30	4		108.0	110.0	109.0	5.7	5.7	5.7
	October 30, 1941	30	7		119.0	124.0	121.0	5.8	6.0	5.9
	November 6, 1941			2	4.2	6.8	5.5			
2814	November 14, 1941	45	1		298.0	308.0	303.0	5.2	6.2	5.7
	November 25, 1942			10	6.2	5.5	5.9			
2801	November 21, 1941	45	3		341.0	342.0	342.0	7.5	7.8	7.7
	November 25, 1941			4	5.9	6.3	6.1	5.6	5.1	5.4
2831	December 9, 1941	45	3		520.0	524.0	522.0	10.1	5.8	
	December 13, 1941			4	19.9	24.6	22.3			
	December 18, 1941			10	6.3	6.7	6.5			
2877	March 5, 1942	45	3		286.0	290.0	288.0	5.9	6.8	6.4
	March 10, 1942			5	7.4	7.4	7.4			

illustrates that this method for determining bound iodine is reasonably reliable.

Nature of iodine compounds precipitated by zinc sulfate and sodium hydroxide

In Figure 1 are illustrated certain experiments with serum precipitable iodine and thyroxine, diiodotyrosine, and after giving desiccated thyroid by mouth. Values represent the iodine in the actual aliquots used, and are the average of duplicate determinations.

In the first 2 experiments, the precipitating agents were added to thyroxine solution in tenth normal alkali. Barred columns at the left represent thyroxine iodine; cross barred columns, filtrate and washings iodine. In the first experiment, only 0.88 of a gamma of iodine was recovered in the precipitable and filtrate iodines, although the original sample contained 1.22 gamma. In the second experiment the water washings, shown at the top of the right column, were found to contain the iodine not recovered in precipitate and filtrate. It is apparent that in the absence of blood serum, prolonged washing with distilled water removed iodine from the precipitate.

In the third and fourth experiments, thyroxine solution was added to duplicate pairs of 2 different sera before precipitation. The amount of iodine in the serum is represented by the open column above the barred thyroxine column. In these 2 experiments, in spite of prolonged washing, total precipitable iodines were 93 and 94 per cent of the sum of the thyroxine and serum iodine.

In the fifth and sixth experiments, an aqueous solution of diiodotyrosine was added to serum before precipitation. Equal aliquots of serum alone were precipitated simultaneously. The iodine was determined in the precipitate and in the combined filtrate and washings of the serum alone and of the serum to which diiodotyrosine was added. The determined diiodotyrosine iodine was only 94.8 per cent of the iodine which should have occurred in the amount of diiodotyrosine (Roche) used. However, the diiodotyrosine had not been repurified or dried. Two different solutions of another sample of diiodotyrosine (Eastman Kodak Company) also gave only 91.5 and 93.5 per cent of the calculated iodine content. In calculating percentage recoveries, it was assumed that the determined iodine was more accurate than the value calculated, on the basis that

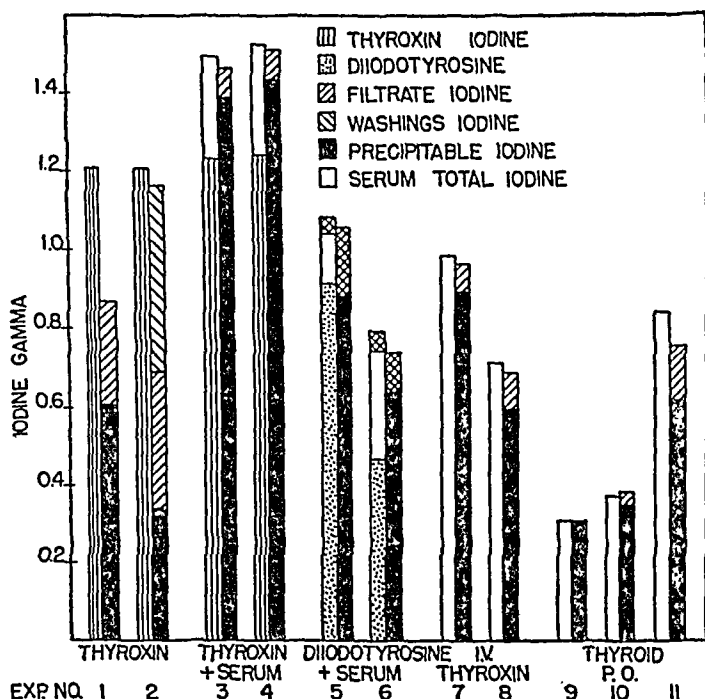


FIG. 1. PRECIPITABLE IODINE RECOVERIES IN *in vitro* AND *in vivo* EXPERIMENTS

Iodines in combined filtrate and washing solutions are represented by superimposing the cross barred symbol for filtrate iodine over the cross barred symbol for washings iodine. In Experiments 5 and 6, the open sections on the left are serum precipitable iodine, and not serum total iodine as in other Experiments.

exactly 58.6 per cent of the weighed diiodotyrosine was iodine.

In experiments 5 and 6, the iodine recovered from precipitate, filtrate, and washings of the serum to which diiodotyrosine had been added, amounted to 95.9 and 93.8 per cent of the sum of the iodine originally in precipitate, filtrate, and washings of the serum, plus the diiodotyrosine which had been added. Analysis of the diagrams in Figure 1 reveals that the major portion of the diiodotyrosine was precipitated with the proteins; but precipitation was not as complete as that of thyroxine. In the precipitates were found only 83.3 and 85.2 per cent of the iodine that should have been expected if all the diiodotyrosine had come down with the precipitable iodine of the serum. In keeping with this, there was more iodine in the filtrate and washings of the treated than of the original serum. Since the quantity of diiodotyrosine added in the 2 experiments differed greatly, while the proportions of bound iodine were the same, the failure of the diiodotyrosine to combine with protein cannot be attrib-

uted to the excessive amounts of the compound used. In addition, it may be seen that far larger amounts of iodine were bound when thyroxine was added. It is impossible to reduce further the quantity of diiodotyrosine and have theoretical recoveries of significant accuracy. What physical or chemical factors enter into this reaction, so that only 83 per cent of the diiodotyrosine iodine was precipitated, are not known.

In the seventh and eighth experiments, serum total, precipitable, and filtrate iodines were determined 2 and 24 hours after intravenous injection of thyroxine solution into a normal male. Precipitable iodines were 94 and 90 per cent of the serum total iodine. In the third, fourth, seventh, and eighth experiments, the filtrate iodines were all within the range found in sera of normal subjects.

In the last 3 experiments, total and precipitable iodines were determined in the sera of 3 different subjects who had been receiving 3, 5, and 15 grains of desiccated thyroid per day by mouth. In the ninth and tenth experiments, after 3 and 5 grains of thyroid, the serum total iodines were still within the normal range and the precipitable iodines were 100 and 92 per cent of the total iodine. In the last experiment, after 15 grains of thyroid, serum total iodine rose to 14 gamma per cent, although prior to thyroid administration the serum total iodine had been 5.6 gamma per cent. When the serum iodine was elevated, the precipitable iodine was only 74 per cent of the total iodine. Whether inorganic iodine rises when large doses of thyroid are given orally cannot be confirmed until another patient can be given large doses of thyroid.

The data in Table I demonstrate that precipitable iodine is not contaminated by inorganic iodine. The third, fourth, seventh, and eighth experiments demonstrate that, in the presence of serum, thyroxine iodine would be included in the precipitable iodine. The fifth and sixth experiments show that at least 83 per cent of diiodotyrosine iodine would also be included in the precipitable iodine.

Precipitable iodine in the serum of hyperthyroid patients

In Table II are the serum precipitable iodines, basal metabolic rates, and treatment of 15 hyper-

SERUM IODINE FRACTIONS IN HYPERTHYROIDISM

thyroid patients. The term thyroidectomy indicates a one stage, and hemithyroidectomy a two stage, operation. The clinical diagnosis of hyperthyroidism was confirmed in all these patients by pathological study of glands and subsequent course after thyroidectomy.

TABLE II
Serum precipitable iodines of hyperthyroid patients

Number, age in years, sex, duration of symptoms in months	Date	Basal metabolic rate	Treatment	Serum iodine		
				Total	Precipitable	Filtrate
		per cent		gamma per cent		
					20.2	1.6
B3055 20 M 24 mon.	November 14, 1941	+35				
A92514 54 F	November 8, 1941 November 10, 1941 November 17, 1941 November 21, 1941 November 22, 1941 December 2, 1941 February 27, 1942	+70 +27 0 +8	Lugol's 15 drops daily Thyroidectomy	17.3	15.5	2.2
B21559 35 F 12 mon.	November 8, 1941 November 12, 1941 November 17, 1941 November 21, 1941 November 25, 1941 December 2, 1941 December 11, 1941	+78 +37 -7	Lugol's 15 drops daily Hemithyroidectomy Hemithyroidectomy	9.6	8.3	0
B23831 62 F 12 mon.	January 22, 1942 January 23, 1942 January 28, 1942 February 3, 1942 February 7, 1942 February 15, 1942 February 16, 1942	+46 +31 +27	Lugol's 15 drops daily Thyroidectomy	11.8	11.5	1.1
B26309 53 F 36 mon.	March 31, 1942 April 2, 1942 April 4, 1942 April 10, 1942 April 16, 1942 April 18, 1942 April 25, 1942 May 15, 1942	+46 +42 +30 +29 +19	Lugol's 15 drops daily Thyroidectomy	4.5	10.7	1.1
B29559 19 F 1 mon.	October 25, 1941 October 26, 1941 November 26, 1941 April 3, 1942 April 17, 1942 April 18, 1942 April 21, 1942 April 23, 1942 April 27, 1942 April 28, 1942 May 1, 1942 May 4, 1942 May 5, 1942 May 6, 1942 May 12, 1942 May 14, 1942 May 23, 1942	+20 +6 +42 +25	Lugol's 7 drops daily Lugol's 15 drops daily Hemithyroidectomy Lugol's 30 drops daily Hemithyroidectomy	10.3	8.0	
A53613 32 F 4 mon.	April 10, 1942 April 11, 1942 April 13, 1942 April 20, 1942 April 22, 1942 April 23, 1942 April 30, 1942 May 1, 1942	+26 +18 +4 +3	Lugol's 15 drops daily Thyroidectomy	11.6	4.7	
B21125 36 F 12 mon.	October 16, 1941 October 17, 1941 October 21, 1941 October 23, 1941 October 24, 1941 October 27, 1941	+34 +16 +11	Lugol's 15 drops daily Thyroidectomy	16.6 116.6	8.6	109.3

TABLE II—Continued

Number, age in years, sex, duration of symptoms in months	Date	Basal metabolic rate	Treatment	Serum iodine		
				Total	Precipitable	Filtrate
		per cent		gamma per cent		
					11.3	
B21360 33 M 12 mon.	October 24, 1941 October 25, 1941 October 27, 1941 October 29, 1941 November 5, 1941 November 11, 1941 November 12, 1941	+30 +24 +26 +9	Lugol's 15 drops daily Thyroidectomy		4.0	
B22239 12 F 1 mon.	November 26, 1941 December 1, 1941 December 2, 1941 December 5, 1941 December 9, 1941 December 12, 1941 December 18, 1941 December 22, 1941 December 29, 1941 January 4, 1942 January 5, 1942 January 6, 1942 January 14, 1942 January 17, 1942 February 12, 1942	+35 +16 +22 +19 +14 +11 +17 -17	Lugol's 3 drops daily Thyroidectomy Lugol's 10 drops daily	16.3 17.2 40.8 6.2	5.9 8.3	
B22457 35 M 18 mon.	December 4, 1941 December 8, 1941 December 10, 1941 December 18, 1941 December 19, 1941 December 24, 1941 December 27, 1941 December 29, 1941 January 1, 1942 January 3, 1942	+50* +45* +20	Lugol's 15 drops daily Hemithyroidectomy Potassium iodide 5 drops daily Hemithyroidectomy	18.6	8.5	
B23562 48 F 3 mon.	January 31, 1942 February 6, 1942 February 18, 1942 March 6, 1942 April 17, 1942 April 27, 1942 April 30, 1942	+35 +22	Lugol's 15 drops daily Thyroidectomy	10.1 9.2	6.1	
B23799 62 M 3 mon.	January 27, 1942 January 28, 1942 January 29, 1942 February 2, 1942 February 9, 1942 February 10, 1942 February 11, 1942 February 15, 1942 February 16, 1942	+50 +21 +15	Lugol's 15 drops daily Thyroidectomy Syrup HI 12 cc. daily	19.4 246 99	9.8 7.3	
Fi 35 M 2 mon.	November 10, 1941 November 11, 1941 January 5, 1942		Lugol's 5 drops daily	9.4 28	8.9	
B25107 53 F 18 mon.	February 25, 1942 March 2, 1942 March 3, 1942 March 10, 1942 March 13, 1942 March 16, 1942 March 20, 1942 March 20, 1942 April 18, 1942 April 21, 1942 April 24, 1942 May 4, 1942 May 8, 1942 May 12, 1942	+25 +31 +42 +18 +26 +30 -4	Lugol's 15 drops daily Thyroidectomy	11.7	9.8 8.9 8.3 10.6 11.8 8.3 6.8	

* Unsatisfactory.

RESULTS

Before the administration of Lugol's solution, measurement of the precipitable, and either the total or filtrate, iodine was made in the serum of the first 6 patients in Table II. All 3 types of iodine were determined in the serum of only

A92514 and B23831. In these 2 instances, the total iodines exceeded the precipitable iodines by 1.8 and 0.3 gamma per cent, while the filtrate iodines were 2.2 and 1.1 gamma per cent respectively. The precipitable iodine of B29589 was 3.6 gamma per cent lower than her total iodine, but the serum in small aliquots was precipitated several days after the blood was taken. Usually serum has been precipitated immediately. However, in this instance contamination from inorganic iodine was feared, and the remainder of the serum was precipitated to rule out this contingency. Since the filtrate iodines were between 1.1 and 2.2 gamma per cent in 5 cases, it has been assumed that, before iodine administration, total iodines would not exceed precipitable iodines by more than about 2 gamma per cent, and therefore in the other cases total iodines alone have been determined before iodine therapy.

Total iodines during Lugol's administration were determined in the sera of only 5 patients (B23831, B21125, B22239, B23799, and Pi). These total iodines were between 28 and 246 gamma per cent. Total iodines of the other patients were undoubtedly similarly elevated.

In all patients, except B29589, Pi, and B25107 who were studied before and after Lugol's, before thyroidectomy, precipitable iodines fell to levels below or just above the maximum normal serum iodine, 8.0 gamma per cent. B29589 had mild symptoms of hyperthyroidism, a basal metabolic rate of plus 20 per cent, and a serum total iodine of 10.3 gamma per cent, in October, 1941. After 31 days, on 7 drops of Lugol's per day, her basal metabolic rate fell to plus 6 per cent, and the precipitable iodine was 8.0 gamma per cent. She was maintained on small doses of Lugol's until 2 weeks before admission to the New Haven Hospital in April, 1942. At the time of admission, her precipitable iodine was 30.1 gamma per cent. After 2 weeks of bed rest and administration of 15 drops of Lugol's daily, she showed some clinical improvement, although the precipitable iodine was 15.6 gamma per cent. A right hemithyroidectomy was followed by a stormy postoperative period. Eight days after removal of the right lobe, the precipitable iodine was still elevated, 12.4 gamma per cent. Two days later, a left hemithyroidectomy was performed. Nine

days after this second stage operation, the precipitable iodine had fallen to 7.5 gamma per cent.

The precipitable iodine of Pi fell only to 8.9 gamma per cent after 49 days of treatment with 5 drops of Lugol's solution daily. At that time, he consulted Dr. Frank Lahey and Dr. Lewis M. Hurxthal. They decided to take him off iodine, but 2½ months later he showed definite clinical evidence of hyperthyroidism. At operation, each lobe of the gland was enlarged to about 3 times normal size. B25107, whose hyperthyroid symptoms increased somewhat and who did not improve even after prolonged Lugol's therapy, had no diminution but a gradual elevation in precipitable iodine. At operation, a large, substernal thyroid was excised.

In contrast with the precipitable iodines of B29589 and B25107, which have just been discussed, are those of B23831 and B23799. After 6 days on Lugol's, the precipitable iodine of B23831 had fallen to 9.4, and after 11 days, to 7.0 gamma per cent. The precipitable iodine of B23799 was 9.8 after 4 days, and 7.3, after 11 days on iodine therapy. It is possible that the precipitable iodines of B21559, B21125 and B22457, which fell only to 9.6, 8.6, and 8.5 gamma per cent, decreased more before thyroidectomy because these precipitable iodines were determined 4, 4, and 9 days before operation. These data indicate that a fall to the normal range of precipitable iodine demonstrated a good response to iodine therapy. Failure of the precipitable iodine to fall after Lugol's administration corresponded with failure of improvement in patient's symptoms. If it is difficult to obtain satisfactory basal metabolisms, the behavior of the precipitable iodine is of diagnostic significance.

Six to 97 days after thyroidectomy, the total or precipitable iodines of 10 patients were determined. The serum total or precipitable iodines of B23831, B26309, B29589, A53613, B21360 and B23799 were within the normal range. A92514, 97 days after thyroidectomy, had a total iodine of 2.0 gamma per cent which was below the normal range, but there were no evident symptoms of thyroid deficiency. B21559, B22239 and B25107, 9, 9, and 10 days after thyroidectomy, had serum precipitable iodines slightly above the normal range. However, subsequent serum total

or precipitable iodines in the sera of B22239 and B25107 were within the normal range. While these postoperative studies have not been made at frequent or prolonged intervals in any patient except A92514, the serum precipitable iodine seems to reach normal concentration about 2 weeks after thyroidectomy.

DISCUSSION

How long after the administration of inorganic iodine the serum total iodine is elevated is a question which determines whether a total or precipitable iodine should be determined. In Table I, 2 euthyroid subjects, 2782, 2798, 2 days after Lugol's solution was stopped, had total iodines of the same magnitude as before iodine was given. One subject, 2831, who had the highest serum total iodine, 522 gamma per cent, has an elevated serum total iodine 4 days after Lugol's was stopped. Unfortunately, her total iodine was not determined again until 6 more days had elapsed. At this time, her total iodine was normal, 6.5 gamma per cent. Riggs, Lavietes and Man have already reported that a hyperthyroid patient, 4 days after omission of 15 drops of Lugol's solution, had a total iodine equivalent to the serum undialyzable iodine while on Lugol's solution-(3). These data indicate that, while 2 to 4 days may suffice for the elimination of inorganic iodine, after large doses more than 4 days but less than 10 days may be required.

The serum iodine values, in Table II, of hyperthyroid patients before treatment are in good agreement with blood iodine levels of untreated hyperthyroid patients, enumerated in an earlier article (1). In blood, the normal range of iodine was found to be 2.4 to 4.2 gamma per cent (1). Determination of the iodine in blood and serum demonstrated that, in the absence of previous iodine therapy, the erythrocytes contained practically no iodine (3, 15). Assuming a normal red cell volume, the normal blood values of 2.4 and 4.2 gamma per cent would be equivalent to serum iodines of 4 to 8 gamma per cent. The blood iodines of 6.4 to 21.9 gamma per cent in the 31 hyperthyroid patients previously described (1) would correspond to serum iodines of 10.7 to 36.0 gamma per cent. The serum iodines of the 15 hyperthyroid patients in Table II, before treatment range from 9.4 to 33.7 gamma per cent.

That all of the 15 hyperthyroid patients in Table II had serum iodines above the normal range confirms the observation in the earlier paper that blood iodine levels are increased in hyperthyroidism. Before treatment, no definite relation existed between the elevation in serum iodine and the severity of exophthalmos. Six patients had no noticeable lid lag or prominence of eyes; exophthalmos was a definite symptom of B21559, B23831, B29589, A53613, B21125, B21360, Pi, and B25107. The average of the initial serum iodines of the patients without exophthalmos was 15.9 and that of the patients with exophthalmos 14.7 gamma per cent. It was reported previously that, even after prolonged hyperthyroidism, the blood iodine was elevated (1). The serum iodine of B26309 is in agreement with this statement. Though this patient had had symptoms of hyperthyroidism for 3 years, her serum iodine was elevated to 10.7 gamma per cent.

SUMMARY AND CONCLUSIONS

A method for separating protein-bound from inorganic serum iodine by precipitation with zinc sulfate and sodium hydroxide has been described. The advantages of this method over previously published techniques have been discussed. The measurement of iodine in the precipitate by a modification of the permanganate acid ashing technique has been described.

The precipitable iodines of 6 euthyroid subjects, who had taken 10 to 45 drops of Lugol's solution per day for 1 to 7 days, agreed within 1.6 gamma per cent with the serum total iodines after Lugol's administration was stopped. Total iodines, before Lugol's, of 2 of these 6 euthyroid subjects differed by only 0.9 gamma per 100 cc. of serum from the precipitable iodines when the patients were on Lugol's. In euthyroid individuals, 2 days after cessation of iodine administration may suffice for the elimination of excess inorganic iodine from the serum, but in 1 subject, between 4 and 10 days were necessary.

In two experiments, after the *in vitro* addition of thyroxine to serum, the precipitate contained 93 and 94 per cent of the thyroxine iodine. After intravenous injection of thyroxine, the precipitable iodines were 94 and 90 per cent of the serum total iodine. Only 83 and 85 per cent of diiodotyrosine iodine were recovered in the precipitate when diio-

dotyrosine solution was added to serum. After oral administration of 3 and 5 grains of thyroid daily, the precipitable iodines were 100 and 92 per cent of the serum total iodine, but after 15 grains daily the precipitable iodine was 74 per cent of the total iodine. It is concluded that this precipitate of serum contains at least 80 per cent of diiodotyrosine iodine and virtually all of the iodine in thyroxin or larger organic compounds of iodine.

Precipitable iodines of 15 hyperthyroid patients have been studied. The clinical diagnosis was confirmed in all these patients by pathological study of the glands. Before treatment with iodine, the serum total or precipitable iodines were between 9.4 and 33.7 gamma per 100 cc., distinctly above the normal range of 4 to 8 gamma per cent. In the first 5 patients, before administration of iodine, the total and precipitable iodines agreed within 2.0 gamma per cent. After Lugol's, before thyroidectomy, precipitable iodines of 11 of 14 patients decreased to concentrations below or just above the maximum normal serum iodine, 8.0 gamma per cent. In 3 patients, whose precipitable iodines did not decrease noticeably, the clinical response to iodine administration was poor. If it is difficult to obtain satisfactory basal metabolisms, the behavior of the precipitable iodine is of diagnostic significance.

Serum total or precipitable iodines of 10 of the 15 patients were within or just above the normal range about 2 weeks after thyroidectomy.

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BIBLIOGRAPHY

1. Riggs, D. S., Gildea, E. F., Man, E. B., and Peters, J. P., Blood iodine in patients with thyroid disease. *J. Clin. Invest.*, 1941, 20, 345.
2. Salter, W. T., *The Endocrine Function of Iodine*. Harvard University Press, Cambridge, Massachusetts, 1940.
3. Riggs, D. S., Laviertes, P. H., and Man, E. B., Investigations on the nature of blood iodine. *J. Biol. Chem.*, 1942, 143, 363.
4. Somogyi, M., A method for the preparation of blood filtrates for the determination of sugar. *J. Biol. Chem.*, 1930, 86, 655.
5. Trevorrow, V., Studies on the nature of the iodine in blood. *J. Biol. Chem.*, 1939, 127, 737.
6. Alpert, L. K., A rapid method for the determination of diodrast-iodine in blood and urine. *Bull. Johns Hopkins Hosp.*, 1941, 68, 522.
7. Lein, A., A rapid method for iodide tolerance determinations. *Endocrinology*, 1941, 29, 905.
8. Boyd, E. M., and Clarke, E. L., The fractionation of cattle blood iodine with alcohol. *J. Biol. Chem.*, 1942, 142, 619.
9. Davison, R. A., and Curtis, G. M., Acetone fractionation of blood and urinary iodine. *Proc. Soc. Exper. Biol. and Med.*, 1939, 41, 637.
10. Davison, R. A., Zollinger, R. W., and Curtis, G. M., The fractionation of the blood iodine. I. Findings in patients with normal thyroid function and with hypothyroidism. *J. Lab. and Clin. Med.*, 1942, 27, 643.
11. McClendon, J. F., and Foster, W. C., Thyroid hormone in blood and tissues in relation to basal metabolic rate. *Endocrinology*, 1941, 28, 412.
12. Bassett, A. M., Coons, A. H., and Salter, W. T., Protein-bound iodine in blood. V. Naturally occurring iodine fractions and their chemical behavior. *Am. J. M. Sc.*, 1941, 202, 516.
13. Salter, W. T., and Bassett, A. M., A physiological interpretation of blood iodine fractions in terms of thyroid function (in 100 cases). *Tr. A. Am. Physicians*, 1941, 61, 77.
14. Riggs, D. S., and Man, E. B., A permanganate acid ashing micromethod for iodine determinations. I. Values in blood of normal subjects. *J. Biol. Chem.*, 1940, 134, 193.
15. Klassen, K. P., Bierbaum, R. L., and Curtis, G. M., The comparative iodine content of whole blood and serum. *J. Lab. and Clin. Med.*, 1940, 26, 365.

SERUM MAGNESIUM IN THYROID DISEASE

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Serum magnesium, like serum calcium, is only partly ultrafiltrable (1). Soffer and his associates, in 1939, reported a consistent increase in the non-ultrafiltrable magnesium of the serum in hyperthyroidism (2). In striking contrast, all of the magnesium of the serum was ultrafiltrable in two patients with myxedema. The present work confirms and extends Soffer's observations.

EXPERIMENTAL PROCEDURES

Blood was drawn, with anaerobic precautions, from subjects in the post-absorptive state. Ultrafiltrate was obtained from the serum by an anaerobic technique, by means of the capsule of Laviertes (3), modified in that the diameter of the effective membrane surface was increased to 3 cm., the cellophane membranes were dried immediately before using by pressing between smooth filter papers, and the filtration pressure was reduced to 28 cm. of mercury. More than 3 cc. of ultrafiltrate were obtained from approximately 10 cc. of serum, in 8 to 10 hours.

One cc. aliquots of serum and ultrafiltrate were delivered into 30 cc. porcelain evaporating dishes. To the ultrafiltrate, 2 drops of saturated sucrose solution were added to prevent subsequent loss by crepitation during ashing. With this exception, serum and ultrafiltrate were treated alike. After adding 1 cc. of 4 N H_2SO_4 to each dish, the dishes were placed on a steam bath for at least 2 or 3 hours, to effect charring. Ashing was accomplished in an electric furnace at 500 to 600° C.

The ash was transferred quantitatively to a 6 cc. volumetric flask as follows. One drop of 4 N H_2SO_4 was rubbed into the ash with a short rounded stirring rod, 1 cc. of water was added with further mixing, and transfer was made into the volumetric flask, facilitated by using petrolatum on the lip of the dish. This was followed by 4 washings with 0.5 cc. water. Three drops of 0.1 per cent brom-cresol green were added to the last washing to detect the presence of acid. An additional washing was used if the indicator was not blue or green. If the necks of the volumetric flasks are 6 mm. in inside diameter, the transfer may be made without the use of a funnel.

To the flask was added 1 cc. of a saturated solution of ammonium oxalate, and dilute NH_4OH sufficient to

develop a full green color with the brom-cresol green (pH 4.2 to 4.4). The volume was then made to 6 cc. with water and mixed by inversion. After standing at least 3 hours, the contents were transferred to a 15 cc. conical centrifuge tube and centrifuged for 10 minutes. Five cc. of the decanted supernatant fluid were transferred to another 15 cc. conical centrifuge tube. One cc. each of 2 per cent $NH_4H_2PO_4$ and concentrated NH_4OH were then added, and the contents mixed. Precipitation was started by scraping the side of the tube with a sharp-tipped fine stirring rod and mixing thoroughly, and was completed by standing at least 8 hours in the refrigerator. It has been learned subsequently that room temperature is satisfactory for this step.

The precipitate was thrown down by centrifugation for 10 minutes, after which the supernatant fluid was decanted and discarded. The precipitate was washed twice with 8 cc. of dilute NH_4OH (2 cc. of concentrated NH_4OH per 100 cc.), by centrifugation and decantation. Two cc. of the wash solution were first run down the side of the tube and the remaining 6 cc. directed forcefully from a fine-tipped pipette onto the surface of the fluid; this prevented loss of precipitate by floating on the surface. Decantation was done rapidly, leaving behind approximately 0.25 cc. each time. An alternative method of washing, adopted after most of the present data were collected, uses a single washing with 7 per cent NH_4OH . Before the first centrifugation, the volume is made to approximately 12 cc. with the wash solution. The single washing is made by running 2 cc. of the wash solution down the side of the tube, followed by approximately 10 cc. directed forcefully at the surface in a fine stream so as to produce frothing. The supernatant fluid is decanted completely, after which the inner lip of the tube is touched with a towel to remove the adherent drop.

The washed precipitate was dried at 95° C., and determined as phosphate by the method of Benedict and Theis (4). After 1 cc. of the acid molybdate reagent had been added to each tube, complete solution of the precipitate, including any adhering to the sides of the tube, was insured by shaking and rotating. Then 5 cc. of water and 1 cc. of hydroquinone were added, and mixed by inversion. Standard tubes containing 0.02 mgm. phosphorus, as KH_2PO_4 , in 5 cc. of water were treated with 1 cc. of the acid molybdate solution, and 1 cc. of hydroquinone sulfite. The tubes were then stoppered lightly with cotton and heated in a boiling water bath for 10 minutes. After cooling and mixing by inversion, the unknowns were compared with the standards by visual colorimetry.

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The calculation is:

$$\frac{24.32}{31.04} \times 0.02 \times \frac{6}{5} \times 100 \times \frac{R}{V} = \text{Mg in milligrams per cent}$$

$$1.88 \frac{R}{V} = \text{Mg in milligrams per cent,}$$

where R is the setting of the standard and V the reading of the unknown.

By this technique, magnesium was recovered from 1 cc. samples of known solutions containing 1.25 to 2.50 mgm. of magnesium as MgSO_4 per 100 cc. of solution, with a maximum error of 0.05 mgm. per cent. These solutions contained calcium and phosphorus in amounts comparable to those in serum. All determinations on serum and ultrafiltrate were made in duplicate, the greatest difference between pairs being 0.03 mgm. per cent. As a further check, in the first 6 ultrafiltrations, magnesium was determined in the concentrated residue, as well as in the serum and ultrafiltrate, and the magnesium of ultrafiltrate plus residue was observed to agree almost exactly with that of an equal volume of serum. As an indication of the reproducibility of results, the determinations were repeated under standard conditions in 3 subjects. The constancy was striking (Table I).

TABLE I

Repeated determinations of bound magnesium in 3 subjects

Subject	Serum	Ultrafiltrate	Bound	
	mgm. per cent	mgm. per cent	mgm. per cent	per cent of total
1 A	2.27	1.75	0.52	23
1 B	2.27	1.73	0.54	24
2 A	1.89	1.88	0.01	1
2 B	1.89	1.87	0.02	1
3 A	1.85	1.61	0.24	13
3 B	1.88	1.64	0.24	13

The arithmetical difference between the concentrations of magnesium in serum and ultrafiltrate has been taken as bound magnesium, following Soffer's example. Bound magnesium might more properly be calculated by subtracting from serum magnesium, not the magnesium in 100 cc. of ultrafiltrate, but the ionized magnesium of the water of 100 cc. of serum. The latter may be approximated by multiplying the magnesium of ultrafiltrate, which is assumed to be completely ionized, by the Donnan ratio for bivalent ions, and again by the water content of the serum. For normal serum, the Donnan ratio would be approximately $(1.05)^2 = 1.10$, and the water content is approximately 93 per cent. Bound magnesium would, then, become serum magnesium — $(1.10 \times 0.93 \times \text{ultrafiltrate magnesium})$ or serum magnesium — $(1.02 \times \text{ultrafiltrate magnesium})$. Practically, since the water content of serum varies relatively little, and since correction for this almost neutralizes that for the Donnan effect, the distribution of values for bound magnesium is not affected by neglect of these corrections. When they are made in the cases of myxedema, bound magnesium becomes —0.03 or —0.04 in all 4 cases of untreated

myxedema, suggesting the possibility that a small amount of the magnesium of ultrafiltrate is unionized.

RESULTS

Bound magnesium has been determined in a group of 14 normal subjects, 9 patients with untreated hyperthyroidism, and 4 patients with untreated myxedema. Blood or serum iodine determination confirmed the clinical diagnoses in these cases, and unmistakable responses to therapy further established them. Total serum magnesium (Figure 1) does not vary significantly in the three groups, but ultrafiltrable magnesium (Figure 2) is distinctly subnormal in hyperthyroidism and

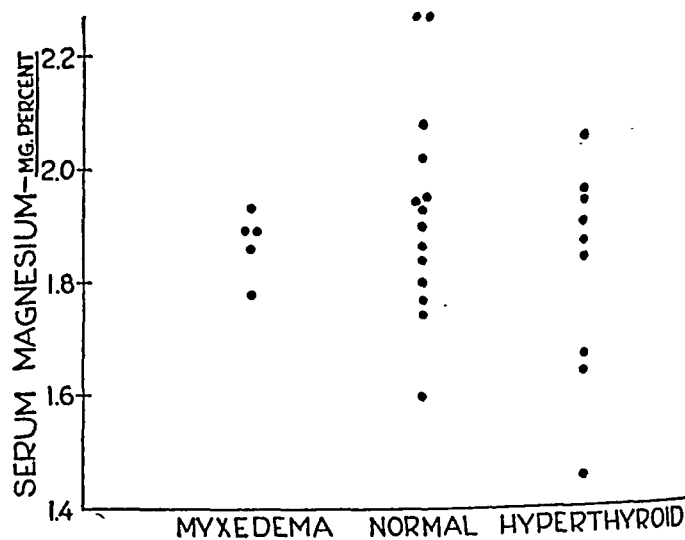


FIG. 1. TOTAL SERUM MAGNESIUM IN NORMAL PERSONS AND PATIENTS WITH THYROID DISEASE

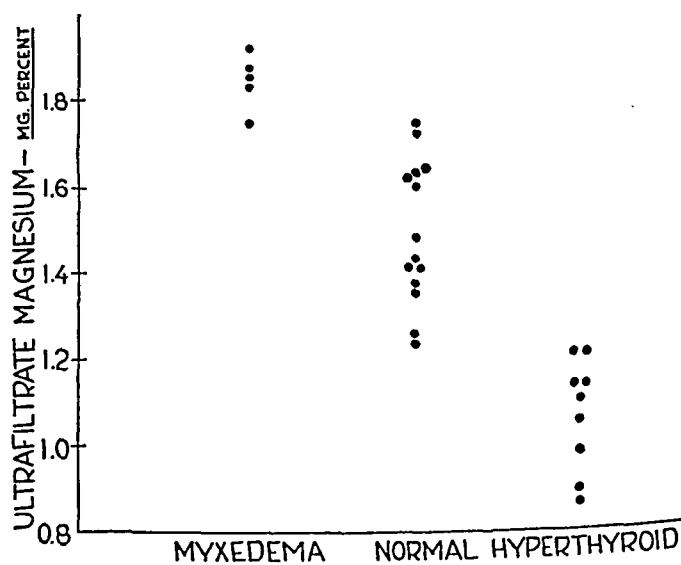


FIG. 2. MAGNESIUM OF ULTRAFILTRATE OF SERUM IN NORMAL PERSONS AND PATIENTS WITH THYROID DISEASE

above normal in myxedema, with consequent striking differences in the bound fraction (Figure 3). In myxedema, bound magnesium is regu-

periods, bound magnesium was above the highest normal value (Table II). In the fifth patient,

TABLE II

Hyperthyroidism after treatment with Lugol's solution

Subject	Magnesium			Basal metabolic rate
	Serum	Ultrafiltrate	Bound	
	mgm. per cent	mgm. per cent	per cent	per cent
1	1.69	1.12	34	+44
2	1.94	1.15	41	+47
3	1.74	1.10	37	+22
4	1.89	1.26	33	+26
5 a	2.22	1.41	37	-5
5 b	2.20	1.48	33	+8
6 a	1.26	1.07	15	+42
6 b	1.56	1.12	28	+27

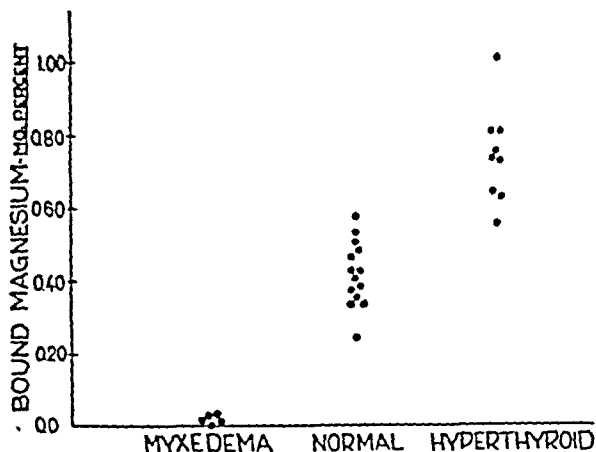


FIG. 3. BOUND MAGNESIUM OF SERUM IN MGM. PER CENT IN NORMAL PERSONS AND PATIENTS WITH THYROID DISEASE

larly absent, and with one exception, and that a minor one, there is no overlapping between the normal and hyperthyroid groups. The differentiation is equally good when bound magnesium is expressed as per cent of the total magnesium (Figure 4). The normal values for bound magnesium fall between 14 and 31 per cent: 12 of the 14 fall within the range 17 to 25 per cent.

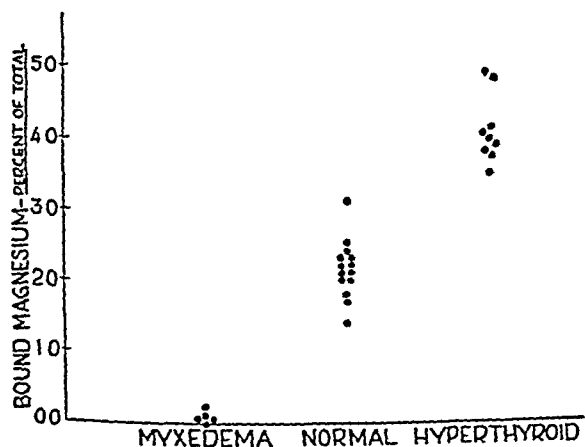


FIG. 4. BOUND MAGNESIUM OF SERUM IN PER CENT OF TOTAL IN NORMAL PERSONS AND PATIENTS WITH THYROID DISEASE

In 5 of 6 patients with hyperthyroidism, studied after the administration of iodine for variable

with mild recurrent thyrotoxicosis controlled symptomatically by continuous therapy with Lugol's solution, bound magnesium was elevated on two occasions when the basal metabolic rate was -5 and +8 per cent, suggesting that the thyrotoxicosis was still active. When therapy was subsequently discontinued for 3 weeks, the basal metabolic rate rose to +17, the basal pulse from 70 to 82, and nervousness and palpitation recurred. This is the only patient in the group who was not subjected to thyroidectomy. The first 4 patients, with severe thyrotoxicosis, were first seen by us after courses of iodine therapy of 1 to 6 weeks duration. The sixth patient, with very severe hyperthyroidism, was first studied 3 days after resuming Lugol's solution, after terminating a long previous course about 2 weeks earlier. Bound magnesium was within normal limits at this time, and again a week later; in both instances, however, ultrafiltrable magnesium was subnormal, as was true of the other hyperthyroid subjects. Unfortunately, this patient was not studied before therapy was started; 8 days after operation, total and bound magnesium were both normal.

Bound magnesium was determined in 7 patients with elevated basal metabolic rate, and some of the other stigmata of thyroid excess, such as nervousness, tremor, and tachycardia, but without hyperthyroidism. It fell within normal limits in all (Table III). Blood or serum iodine values were normal in every instance, and at least one course of Lugol's solution was given to each

TABLE III
Hypermetabolism without hyperthyroidism

Subject	Magnesium			Basal metabolic rate
	Serum	Ultrafiltrate	Bound	
	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>per cent</i>	<i>per cent</i>
1	1.94	1.56	20	+48
2	1.58	1.22	23	+16
3	2.07	1.71	17	+46
4	2.12	1.65	22	+28
5	1.77	1.21	32	+43
6	1.73	1.38	20	+9
7	1.82	1.38	24	+29
8	1.74	1.49	14	+19

patient without effect on symptoms or metabolic rate, independent evidence that hyperthyroidism was not responsible for the elevated basal metabolism. Two of these patients had marked Parkinsonism, and all of the others had considerable vasomotor and nervous instability.

Three patients with myxedema, adequately controlled by the administration of 2 grains of desiccated thyroid daily, had normal amounts of bound magnesium in their sera. In all of 7 hyperthyroid patients, bound magnesium, which was elevated before thyroidectomy, returned to or below normal limits after operation.

DISCUSSION

Our data give the following indications concerning bound magnesium: (1) It varies over a fairly narrow range in normal subjects. (2) It is consistently above the normal range in hyperthyroidism, falling towards normal under treatment with iodine, and to normal after thyroidectomy. (3) It is entirely lacking in myxedema, returning to normal, however, under treatment with thyroid substance. (4) It is normal in patients with abnormal basal metabolism unassociated with thyroid disease. The significance of these observations in relation to diagnosis is self-evident. A recent observation of Soffer (5) that bound magnesium may be normal in some patients with hyperthyroidism, especially when the disease is mild or of long duration, finds no counterpart in our limited experience, although symptoms had been present for more than 2 years in 7 of our cases, and the disease was fairly mild in 3 of these.

Our normal values for bound magnesium are significantly higher and less disperse than those of Soffer. Differences in technique suggest several possible explanations for this discrepancy. For ultrafiltration, Soffer used a pressure of 80 lbs. of nitrogen per square inch. Observations by Flexner (6) indicate that, under such high pressure, ultrafiltrate is not in dialysis equilibrium with the residue, and the discrepancy is in the proper direction to explain the discrepancy between Soffer's data and ours. Watchorn and McCance (1), using low pressure ultrafiltration, observed values comparable to ours. Changes in volume by evaporation or condensation of water during ultrafiltration, possible when gas under high pressure is used, were avoided by our anaerobic technique. Our use of ashes, instead of trichloroacetic acid filtrates, for analysis eliminates possible errors incurred in precipitation of proteins. Our values for total serum magnesium, in distinction to bound magnesium, are approximately 25 per cent lower than Soffer's; since we recovered known solutions quantitatively, this suggests the possibility that their precipitates were impure or incompletely washed.

At present, one can only speculate on the nature of the bound magnesium. Failure to pass through a cellophane membrane indicates that it is incorporated in, or associated with, the colloids of the serum. Unlike calcium, it is not combined with protein in general, being completely absent in myxedema, in which serum proteins tend to be high. Since serum fats, phospholipids, and cholesterol are also elevated in myxedema, it seems improbable that these are responsible for the binding of magnesium. This suggests that it is associated with a specialized protein such as an enzyme or hormone. A rough correlation exists between serum hormonal iodine and bound magnesium in normal subjects and untreated patients, and the increase of each above normal in hyperthyroidism is approximately the same. If a significant correlation between bound magnesium and serum iodine can be established, the magnesium need still not be part of the circulating thyroid hormone, but may rather be associated with the complex in which the hormone functions, possibly an enzyme system. Thus, for example, magnesium is known to serve as an activator for at

least some of the enzymes and coenzymes involved in exchanges of phosphate in intracellular intermediary metabolic processes. It is essential for the activation of adenosinetriphosphate, carboxylase, certain dehydrogenases, and kidney phosphatase. Soffer (2) has observed that thyroglobulin does not bind magnesium *in vitro*, and that there is no immediate rise in bound magnesium after the injection of either thyroglobulin or thyroxine into normal dogs. There is a transitory delayed increase after the thyroglobulin, and a prolonged delayed increase after thyroxine, indicating that these substances must undergo modification, or initiate change in other systems, before affecting bound magnesium.

Bound calcium is apparently physiologically inactive, owing its existence only to the chemical affinity of the serum proteins for calcium, the latter being dependent on the resultant of equilibria between the plasma and the gastrointestinal tract, bones, and kidneys. Bound magnesium, in contrast, is probably physiologically active, in a manner quite different from the ultrafiltrable fraction, which, like ultrafiltrable calcium, is presumably essentially completely ionized. The effect of magnesium on excitability of neuromuscular mechanisms is undoubtedly an ionic one. Any explanation of the effect of hyperthyroidism upon serum magnesium, must account not only for the increased binding of magnesium but also for the diminished concentration of ionized magnesium. Ionized magnesium must be the result of the equilibria between serum and gastrointestinal tract, bones, intracellular ionized magnesium, and the kidneys. It is, therefore, difficult to conceive of a mechanism by which increase of bound magnesium results in compensatory decrease in ultrafiltrable magnesium.

Preliminary observations by Dr. Francis P. Vose, in this department, demonstrate an excessive post-absorptive renal excretion of magnesium in hyperthyroidism. Since the concentration of magnesium in ultrafiltrate of serum, and thus presumably in glomerular filtrate, is subnormal in hyperthyroidism, this increased renal excretion must indicate a very large increase and volume of glomerular filtrate, or a diminution of tubular reabsorption of magnesium. The latter is more probably the case since the increases in volume of

glomerular filtrate reported in hyperthyroidism (7, 8) are not sufficiently high to explain the increase in urinary magnesium. In any event, the excessive urinary excretion must be responsible, at least in part, for the subnormal concentration of ultrafiltrable magnesium in the serum of hyperthyroid subjects. It cannot explain the extraordinary increase in bound magnesium, which leaves total magnesium normal.

The therapeutic applications of magnesium in thyroid disease are as yet undetermined. One investigator has claimed the regression of symptoms of Graves' disease and a reduction of basal metabolism following injections of magnesium (9). We have not been able to duplicate these results.

SUMMARY AND CONCLUSIONS

A correlation between thyroid function and the state of the serum magnesium has been observed, confirming previous work.

Refinements of technique are described.

In 14 normal subjects, the non-ultrafiltrable, or bound, fraction of the serum magnesium was 17 to 31 per cent of the total. In each of 9 proved untreated cases of hyperthyroidism, bound magnesium exceeded these values, and ultrafiltrable magnesium was subnormal. In 4 patients with myxedema, all of the magnesium of the serum was ultrafiltrable. After therapy of thyroid dysfunction, bound and ultrafiltrable magnesium return to normal. In 8 patients with hypermetabolism without hyperthyroidism, bound magnesium was normal.

The possible relation of bound magnesium to the circulating thyroid hormone or to enzyme systems is discussed.

Attention is directed to the anomalous reciprocal changes of bound and free magnesium.

BIBLIOGRAPHY

1. Watchorn, E., and McCance, R. A., Inorganic constituents of cerebrospinal fluid: The ultrafiltration of calcium and magnesium from human sera. *Biochem. J.*, 1932, 26, 54.
2. Soffer, L. J., Dantes, D. A., Grossman, E. B., Sobotka, H., and Jacobs, M. D., Ultrafiltrable magnesium in hyperthyroidism. *J. Clin. Invest.*, 1939, 18, 597.
3. Laviates, P. H., Anaerobic ultrafiltration. *J. Biol. Chem.*, 1937, 120, 267.
4. Benedict, S. R., and Theis, R. C., A modification of

- the molybdc method for the determination of inorganic phosphorus in serum. *J. Biol. Chem.*, 1924, **61**, 63.
5. Soffer, L. J., Cohn, C., Grossman, E. B., Jacobs, M. D., and Sobotka, H., Magnesium partition studies in Graves' disease and in clinical and experimental hypothyroidism. *J. Clin. Invest.*, 1941, **20**, 429.
 6. Flexner, L. B., A thermodynamic analysis of ultrafiltration. The ultrafiltration of sucrose and colloidal solutions. *J. Biol. Chem.*, 1937, **121**, 615.
 7. Medes, G., and Herrick, J. F., Blood flow to the kidney and creatinine clearance. *Proc. Soc. Exper. Biol. and Med.*, 1933, **31**, 116.
 8. Winkler, A. W., and Parra, J., The measurement of glomerular filtration. Creatinine, sucrose and urea clearances in subjects without renal disease. *J. Clin. Invest.*, 1937, **16**, 859.
 9. Heuber, E. F., Ueber die Beeinflussung von Hyperthyreosen durch Magnesiumglutaminat. *Wien. klin. Wchnschr.*, 1939, **52**, 932.

PROLONGED WATER DEPRIVATION IN THE DOG¹

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Evidence of intracellular as well as of extracellular water depletion was obtained during periods of water deprivation in some experiments on pyloric obstruction in dogs. The object of this control study was to observe the exchanges of body water and salts as conditioned by the strict deprivation of water.

Kerpel-Fronius (1) showed that in fasting rabbits deprived of water, the concentration of sodium in serum rose and the ratio of potassium to nitrogen excreted exceeded that in cell fluid. These findings were interpreted as indicating a loss of both intracellular and extracellular water. Nadal, Pedersen, and Maddock (2) obtained somewhat similar evidence in human subjects. Both of these papers emphasized the contrast between the intra- and extracellular water depletion that arose from a primary loss of pure water, and the predominantly extracellular depletion where fluids containing salt are lost. But the distinction was not made between the cell water released with potassium and that lost on an osmotic basis.

Darrow and Yannet (3) and Hastings and Eichelberger (4, 5) have shown that water is freely diffusible across cell membranes and that it shifts according to the osmotic concentration of the bases which are restrained on either side. Evidence has accumulated, however, that these bases are not entirely restrained. The factors which control their movements are not clear, but such movements must ultimately control the distribution of water between the two phases. Sodium is known to displace intracellular potassium under certain nutritional (6) and hormonal (7) stimuli. But the conditions are still obscure under which sodium and potassium cross the cell boundary in response to changes of ionic concentration and of water volume.

The following experiments in prolonged water deprivation constitute an attempt to define the range of variation of total ionic concentration and

of volume of body fluids, to observe the associated renal reactions, and to differentiate the mechanisms by which cell water is released.

METHODS

In *serum*, the concentration of chloride was determined by the method of Hald (8), sodium and potassium by the method of Hald (9), sodium sulfocyanate by the method of Crandall and Anderson (10) as adapted to the photoelectric colorimeter by Elkinton and Taffel (11), and water content by the difference between wet and dry weights. The whole blood non-protein nitrogen was measured by the micro-kjeldahl technique.

In *urine*, chloride was determined by the Volhard-Harvey titration (12), sodium by the method of Butler and Tuthill (13), potassium by the method of Hald (9), sodium sulfocyanate by Elkinton and Taffel's (11) modification of the method of Crandall and Anderson (10), and total nitrogen by macro-kjeldahl.

EXPERIMENTAL PROCEDURE

Four normal dogs were deprived of water and food for periods of 11 to 20 days. During these periods, the balances of water, chloride, sodium, potassium, and nitrogen were measured. From these balances, and from studies of the distribution of sodium sulfocyanate, the exchanges of water and salt in the animals were calculated.

The dogs were kept in metabolic cages and all urine was collected. No fecal analyses were made since the amount of feces obtained was insignificant, owing to the starvation. The weight change, however, was corrected for the 2 small stools passed by 1 dog, as well as for losses of tissue and blood in surgical procedures and blood analyses, respectively. The dogs were weighed at the beginning and end of each period on scales with an accuracy of ± 10 grams.

Blood for analysis was taken from the jugular vein; that portion for determination of the non-protein nitrogen was oxalated, the rest was allowed to separate into clot and serum. The urine collected was preserved with thymol at 5° C.

The distribution volume of sulfocyanate was determined several times in 3 of the 4 dogs. A 1 per cent solution of sodium sulfocyanate was injected intravenously from a calibrated burette and the serum concentration and urinary excretion determined at the end of a 4-hour interval (11). To insure complete urinary recovery, the animal was catheterized and the bladder was washed 3 times with normal saline solution.

Two of the dogs (Dogs 6 and 8) had laparotomies and 1 (Dog 7) received anesthesia, as controls for the operative transection of the pylorus in the previous experiments. Each dog received a small infusion of 5 per cent glucose and 0.9 per cent sodium chloride solution postoperatively.

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Dog 6 was rehydrated on the twentieth day, Dog 7 died on the eleventh day, and Dogs 8 and 9 were killed on the fifteenth day when Dog 8 was moribund.

CALCULATIONS

Total water changes (ΔW) were calculated from weight changes corrected for solids lost and the metabolic mixture, as estimated according to the method of Newburgh (14, 15):

$$\Delta W = \Delta Wt.' + (C + 0.49P + F), \quad (1)$$

where the protein burned (P) was calculated from the nitrogen excretion, the carbohydrate burned (C) was assumed to equal the carbohydrate given, and the fat burned was calculated in the following ways. Method A: The total caloric expenditure of the fasting dog, as determined by direct calorimetry by Anderson and Lusk (16), was approximately 2.0 calories per kilogram per hour, decreasing 1.5 per cent per day. The fat burned was calculated from the total calories:

$$F = (\text{Total cal.} - P + C \text{ cal.})/9.3. \quad (2)$$

Method B: The fat burned was calculated from the insensible weight loss (IL) by Laviertes' formula (17):

$$F = (IL - 2.12C - 1.69P)/3.78. \quad (3)$$

ΔW was calculated from both figures for fat burned and the average taken (Table III).

Extracellular volume changes (ΔE) were measured directly by the distribution of sodium sulfocyanate (11):

$$E_{SCN} = \frac{\text{Net retention NaSCN in mgm.}}{\text{Serum concentration in mgm. per liter}} \quad (4)$$

and from the chloride and sodium balances:

$$E_{Cl_2} = \frac{(E_1 Cl_1) + b_{Cl_1}}{Cl_2}, \quad (5)$$

$$E_{Na_2} = \frac{(E_1 Na_1) + b_{Na_1}}{Na_2}, \quad (6)$$

where:

b_{Cl_1} and b_{Na_1} = balances of Cl and Na,

E_1 = initial extracellular fluid volume,

Cl_1 and Cl_2 = initial and final concentrations of Cl in extracellular water,

Na_1 and Na_2 = initial and final concentrations of Na in extracellular water.

In this calculation, it was assumed that the initial extracellular fluid volume (E_1) equalled either 27 per cent (18) of the body weight (Dog 6), or that the chloride and sodium spaces were identical with the sulfocyanate space (Dogs 7, 8, and 9). The concentrations of Cl, Na, and K in extracellular water (ECW) were calculated from the serum concentrations (s) by the use of a Donnan factor of 0.95:

$$Cl_{ECW} = Cl_s/(W_s \times 0.95), \quad (7)$$

$$Na_{ECW} = (Na_s \times 0.95)/W_s, \quad (8)$$

$$K_{ECW} = (K_s \times 0.95)/W_s, \quad (9)$$

where W_s = grams of water in 100 grams of serum.³

³ Had W_s been calculated per 100 cc. of serum, the values for ΔE and ΔI in Table V would be changed by not more than 5 per cent.

In Dogs 8 and 9, the distribution volume⁴ of the radioactive isotope of Cl ($E_{Cl^{38}}$) was determined at the end of the experiment. For further calculations,

$$\Delta E = E_2 - E_1, \quad (10)$$

where E_2 was taken to be the average of E_{Cl_2} and E_{Na_2} .

The change in intracellular water volume (ΔI) was estimated in two ways. In the first method, ΔI was taken to equal the difference between the total water change (ΔW) and the extracellular water change (ΔE):

$$\Delta I_I = \Delta W - \Delta E. \quad (11)$$

In the second method, ΔI was calculated as the sum of 3 decrements: (1) cell water lost with the consumption of protein during fasting (ΔI_P), (2) cell water lost with potassium released in excess of nitrogen ($\Delta I_K'$), and (3) cell water lost by osmotic shift ($\Delta I_{\Delta B}$):

$$\Delta I_{II} = \Delta I_P + \Delta I_K' + \Delta I_{\Delta B}. \quad (12)$$

The formulae for the first 2 decrements are derived from the ratios of water and potassium to protein in the cell fluid of skeletal muscle as determined in 20 normal dogs by Hastings (4):

$H_2O = 0.27$ liters per 100 grams protein,

$K = 38$ m. Eq. per 100 grams protein,

assuming 92.6 per cent of the solids to be protein (Darrow (19)). Thus:

$$\Delta I_P = 2.7 \times P, \quad (13)$$

where P = protein burned in kilograms, and

$$\Delta I_K' = b_K'/B_2, \quad (14)$$

where B_2 = the total concentration of ionically active base in the cell (see below),

$$b_K' = b_{KI} - b_{KP} = \text{balance of excess K}, \quad (15)$$

$$b_{KP} = 380 \times P = \text{balance of K lost with protein}, \quad (16)$$

$$b_{KI} = b_K - b_{KE} = \text{balance of intracellular K}, \quad (17)$$

$$b_{KE} = (K_{ECW_2} \times E_2) - (K_{ECW_1} \times E_1) \\ = \text{balance of extracellular K}, \quad (18)$$

b_K = total balance of K.

The concentration of potassium in serum was determined on old samples and the results appear slightly elevated. This does not, however, affect the correction for extracellular K (b_{KE}). The high value at the end of the experiment in Dog 7 is undoubtedly due to the blood being drawn after death. As serum was not available for analysis in Dog 6, a value for K_{ECW} was assumed.

The calculation of the third decrement ($\Delta I_{\Delta B}$) involves an assumption that the total concentration of ionized base in cell water is equal to that in extracellular water, the latter value being taken to be 10 m. Eq. per liter greater than the sodium concentration. The calculation also required a value for the initial intracellular water volume. This was derived by arbitrarily assuming the total body water to be 65 per cent of the body weight. Published

⁴ Determined with the collaboration of Drs. A. W. Winkler and A. J. Eisenman.

values of water content of whole dogs, as determined by desiccation, range from 55 per cent for fat dogs to 68 per cent for lean dogs (18, 20). The dogs used were lean. Thus:

$$\Delta I_{AB} = -I_1 \Delta B/B_1, \quad (19)$$

where $I_1 = (0.65 \text{ Wt.}) - E_1, \quad (20)$

$$\Delta B = B_2 - B_1, \quad (21)$$

$$B = \text{Na}_{\text{ECF}} + 10 \text{ m. Eq. per liter.} \quad (22)$$

Balances of water, chloride, and sodium include corrections for losses in blood drawn for analysis (Table I). The nitrogen balance (b_N') consists of the urinary excretion (b_N) corrected for change in non-protein nitrogen content in the body fluids. This correction was made by approximating the total body water from the body weight; the correction was for the most part negligible.

RESULTS

The concentration of sodium in serum rose in each dog, indicating an increase in total ionic concentration in the body fluids (Table II). The extent of the increase was 15 to 41 m. Eq. per liter. The larger increases were observed in the dog that died and in the dog that became moribund.

The extracellular water loss varied from 20 per cent to 38 per cent of the initial extracellular volume (Table IV). In each experiment, it was exceeded by the calculated total water loss, indicating that a substantial portion of the water lost came from the cells (Table V). Intracellular water loss as calculated by difference (ΔI_I) varied from 30 to 55 per cent of the initial volume. In 3 of the 4 dogs, the percentage water losses from each of the 2 phases were approximately equal.

The total amount of water lost from the cells could be differentiated into three processes (Table V, Figure 1). Cell water was lost with protein consumed as the result of fasting (ΔI_P). The rise in total ionic concentration as measured by the concentration of sodium in serum accounted for a second decrement of cell water on a purely osmotic basis (ΔI_{AB}). The potassium excretion was considerably in excess of the amount calculated to accompany the nitrogen excretion. The passage of extra potassium from the cell released a third decrement of cell water (ΔI_K). The sum of these decrements (ΔI_{II}) was in reasonable

TABLE I
Exchange of water, electrolytes, and nitrogen
Quantities expressed per individual period.

Dog	Period	Intake parenteral			Output							
					Urine					Blood *		
		H ₂ O	Na	Cl	H ₂ O	Na	Cl	K	N	H ₂ O	Na	Cl
6	days	cc.	m. Eq.	m. Eq.	cc.	m. Eq.	m. Eq.	m. Eq.	grams	cc.	m. Eq.	m. Eq.
	1 to 4	600†	46.5	46.5	430	34.0	34.8	19.7	5.66	24	3.8	2.1
	5 to 8				130	11.5	17.4	22.4	7.61	16	2.5	1.4
	9 to 12	20†	2.5		55	0.4	8.6	17.6	5.13	16	2.5	1.4
	13 to 16				25	0.1	1.5	9.0	2.80	24	3.7	2.1
	17 to 20	20†	2.5		25	0	0.8	12.5	2.79	26	4.3	2.6
7	1 to 2	105†	3.9	3.9	255	33.2	23.6	21.4	4.24	25	1.7	1.3
	3 to 4				78	9.6	6.9	21.6	4.37	27	2.0	1.5
	5 to 6	20†	2.5		72	3.5	3.5	16.2	3.92	33	2.5	2.0
	7 to 11				65	1.0	5.4	16.5	3.55	29	2.3	1.7
8	1 to 2	209†	11.6	11.6	1155§	74.0	54.2	33.3	6.58	25	1.8	1.2
	3 to 4				106	16.8	12.1	33.8	5.95	29	2.1	1.5
	5 to 6	40†	4.9		170	21.2	23.8	38.2	8.91	37	2.7	2.0
	7 to 12				150	10.3	9.4	54.5	13.97	29	2.1	1.6
	13 to 15	30†	2.5	2.0	45	1.8	0	21.6	3.38	38	3.2	2.6
9	1 to 2	200†	11.6	11.6	479	52.7	39.0	33.6	6.35	25	1.8	1.3
	3 to 4				60	3.1	1.4	19.6	3.55	29	2.1	1.5
	5 to 6	40†	4.9		174	19.0	14.1	48.3	8.40	39	3.0	2.3
	7 to 12				375	22.8	16.3	74.8	19.68	29	2.1	1.6
	13 to 15	30†	2.5	2.0	139	0.2	2.2	20.7	9.35	41	3.2	2.5

* Blood drawn for analyses.

† Postoperative and control infusions.

‡ NaSCN given for measurement of volume of distribution.

§ Unexplained diuresis, possibly due to pyrogens in infused fluid.

TABLE II
Analyses of blood and serum
Time at end of day indicated.

Dog	Time	Blood NPN	Serum			
			Na	Cl	K	H ₂ O
	day	mgm. per cent	m. Eq. per liter	m. Eq. per liter	m. Eq. per liter	per cent
6	0	24	143.8	108.6		94.8
	4	35	148.0	115.2		95.0
	8	31	150.2	116.0		94.7
	12	33	147.6	118.7		94.8
	16	31	157.0	123.2		94.5
	20	33	159.6	129.9		94.7
7	0	28	145.1	110.1	6.51	92.6
	6	35	161.3	125.1	5.72	91.2
	11*	270	186.1	129.5	9.38	88.0
8	0	35	147.1	102.4	5.62	93.3
	6	53	151.4	111.5	5.07	91.1
	12	53	165.1	125.4	5.17	89.3
	15†	152	170.2	133.0	6.44	89.3
9	0	27	146.8	106.9	6.19	91.6
	6	27	151.5	113.4	5.52	90.4
	12	31	156.1	123.6	6.90	90.6
	15	34	162.2	127.9	5.18	90.6

* Died. Blood for analysis taken 20 to 40 minutes postmortem.

† Moribund.

agreement in 3 of the 4 dogs, with the difference between the extracellular and the total water loss

as calculated from the weight change, (ΔI_1) (Figure 1).

In the urine, the concentrations of sodium and of chloride diminished to the vanishing point, whereas the potassium concentration steadily increased, (Figure 2). Non-protein nitrogen of blood did not rise until the terminal stages, and the specific gravity of the urine remained high.

DISCUSSION

Assumptions. The experimental results cast some light on the assumptions used in the calculations. In each experiment, the extracellular volume loss as calculated by the chloride balance exceeded that by the sodium balance and by sulfocyanate distribution, (Table IV). In the dog muscles analyzed by Hastings (4), the sodium space was 16 per cent greater than the chloride space. If ΔE_{Cl} is calculated from an initial volume which is 20 per cent smaller than the sulfocyanate space, the agreement between ΔE_{Cl} , ΔE_{Na} , and ΔE_{SCN} is closer.

The assumptions that intracellular and extracellular ionized base concentrations are equal and that potassium excretion measures the loss of ionized base from the cells, appear justified by the fairly good agreement in 3 dogs between the

TABLE III
Estimation of metabolic mixture

Data are expressed cumulatively from the beginning of the experiment to the end of the day indicated.

Dog	Time	Weight	Change of weight	Nitrogen	Protein	Carbo- hydrate	Method A		Method B	
							Total calories	(2) * fat	Insensible weight loss	(3) fat
	day	kgm.	kgm.	grams	grams	grams		grams	kgm.	grams
6	0	7.54								
	4	6.85	-0.69	6.2	39	15	1360	123	0.84	197
	8	6.28	-1.26	13.6	85	15	2633	239	1.26	287
	12	5.82	-1.72	18.8	118	15	3820	411	1.67	380
	16	5.36	-2.18	21.6	135	15	4920	528	2.08	462
	20	4.92	-2.62	24.4	153	15	5932	564	2.49	582
7	0	7.07								
	6	5.50	-1.55	12.8	80	4	1907	168	1.18	274
	11	4.76	-2.29	22.4	140	4	3454	300	1.83	419
8	0	13.87								
	6	11.20	-2.61	23.0	144	6	3750	337	1.34	287
	12	9.78	-4.02	37.0	231	6	7140	663	2.57	575
	15	9.29	-4.50	44.8	280	6	8700	809	2.99	663
9	0	15.71								
	6	13.72	-1.90	18.3	115	6	4244	403	1.33	298
	12	12.30	-3.27	38.3	239	6	8080	761	2.30	498
	15	11.69	-3.87	47.8	299	6	9841	924	2.75	588

* Number at head of column indicates equation in text (Calculations) from which data are derived.

TABLE IV
Calculation of extracellular fluid volume
Time at end of day indicated.

Dog	Time	(8) † Na _{ECW}	(7) Cl _{ECW}	(4) E _{SCN}	(6) E _{Na}	(5) E _{Cl}	* E _{Cl} †
	day	rr. Eq. per liter	rr. Eq. per liter	liters	liters	liters	liters
6	0	144.0	120.4		2.04	2.04	
	4	148.0	126.7		2.04	2.02	
	8	150.9	128.9		1.91	1.84	
	12	148.0	131.8		1.94	1.72	
	16	157.8	137.3		1.80	1.63	
	20	160.1	144.3		1.76	1.53	
7	0	148.8	125.2	2.12	2.12	2.12	
	6	168.2	144.3	1.66	1.61	1.60	
	11	200.2	155.0		1.33	1.45	
8	0	149.8	115.6	3.90	3.90	3.90	
	6	158.1	128.9	3.09	3.04	2.86	
	12	175.8	147.9		2.67	2.42	
	15	181.1	156.7	2.53	2.59	2.28	2.60
9	0	152.1	122.7	3.90	3.90	3.90	
	6	159.2	132.2	3.60	3.32	3.26	
	12	163.8	143.7		3.07	2.88	
	15	170.2	148.7	3.01	2.95	2.76	2.90

* Measured by means of radioactive chloride, Cl³⁵.

† Number at head of column indicates equation in text (Calculations) from which data are derived.

intracellular water loss calculated by difference from the weight loss (ΔI_T) and the sum (ΔI_{II}) of the decrements ΔI_P , $\Delta I_{K'}$, and ΔI_{AB} , which were calculated from these assumptions. In 1 dog (Dog 6), ΔI_T greatly exceeded ΔI_{II} . The experiment was performed in the heat of midsummer. If the discrepancy were due to the provision of more water of oxidation than was calculated, the total heat production would have to be doubled and the proportion of heat lost by vaporization of water reduced by one-half. This is a less likely explanation than that the dog sweated and the salt recoveries were too low.

Exchange of bases. In water deprivation, the obligatory vaporization of water for the removal of heat entails a loss of water without salt. Hypertonicity of body fluids is the result in the absence of a compensatory salt excretion. Salt excretion, as well as the distribution of the water loss between the 2 phases of body water, is conditioned by the behavior of the extra- and intracellular bases. Study of base exchange is facilitated in the dog by the usual absence of an extrarenal route for the elimination of salt.

The excretion of sodium, as well as of chloride,

TABLE V
Allocation of water loss

Data are expressed cumulatively from the beginning of the experiment to the end of the day indicated.

Dog	Time	(1) * ΔW	(10) ΔE	(11) ΔI_T	(13) ΔI_P	(19) ΔI_{AB}	(14) $\Delta I_{K'}$	(12) ΔI_{II}
	day	liters	liters	liters	liters	liters	liters	liters
6	4	-0.50	-0.01	-0.49	-0.10	-0.07	-0.03	-0.20
	8	-0.95	-0.17	-0.78	-0.23	-0.12	-0.06	-0.41
	12	-1.26	-0.21	-1.05	-0.32	-0.07	-0.09	-0.48
	16	-1.61	-0.33	-1.28	-0.36	-0.24	-0.10	-0.70
	20	-1.96	-0.40	-1.56	-0.41	-0.27	-0.12	-0.80
7	6	-1.29	-0.52	-0.77	-0.22	-0.26	-0.14	-0.62
	11	-1.86	-0.73	-1.13	-0.38	-0.60	-0.11	-1.09
8	6	-2.23	-0.95	-1.28	-0.39	-0.24	-0.26	-0.89
	12	-3.29	-1.36	-1.93	-0.62	-0.72	-0.34	-1.68
	15	-3.62	-1.47	-2.15	-0.76	-0.83	-0.36	-1.95
9	6	-1.49	-0.61	-0.88	-0.31	-0.26	-0.31	-0.88
	12	-2.52	-0.93	-1.59	-0.65	-0.44	-0.47	-1.56
	15	-2.97	-1.05	-1.92	-0.81	-0.63	-0.41	-1.85

* Number at head of column indicates equation in text (Calculations) from which data are derived.

practically ceased under the conditions of our experiment. The high specific gravity of the urine and the adequate excretion of non-protein nitrogen are evidence against failure of renal function. Sodium and chloride must have been almost completely reabsorbed in the tubules despite the increasingly high concentration of these substances in the serum, a phenomenon noted by Kerpel-Fronius.

Although these animals were completely deprived of water, water was provided for vaporization and for an adequate excretion of urine. To provide this amount of water, the extracellular reservoir would have been completely exhausted if cell water had not been made available. A larger sodium excretion would increase the extracellular water loss. By retention of sodium, less of such water was lost, and the rise in sodium concentration made, on an osmotic basis, a fraction of cell water available (ΔI_{AB}). In addition, cell water was released, not only as a result of the fasting state (ΔI_P), but also as a result of the washing out of excess potassium ($\Delta I_{K'}$). Consequently, the cells shared both the hypertonicity and the reduction of volume. Under a compulsion to lose base, the preferential excretion of potassium over sodium increased intracellular and decreased extracellular water loss.

Schwartz, Smith, and Winkler (21) have observed a similar phenomenon following the injection of a combined solution of sodium chloride and sodium sulfate. A preferential excretion of

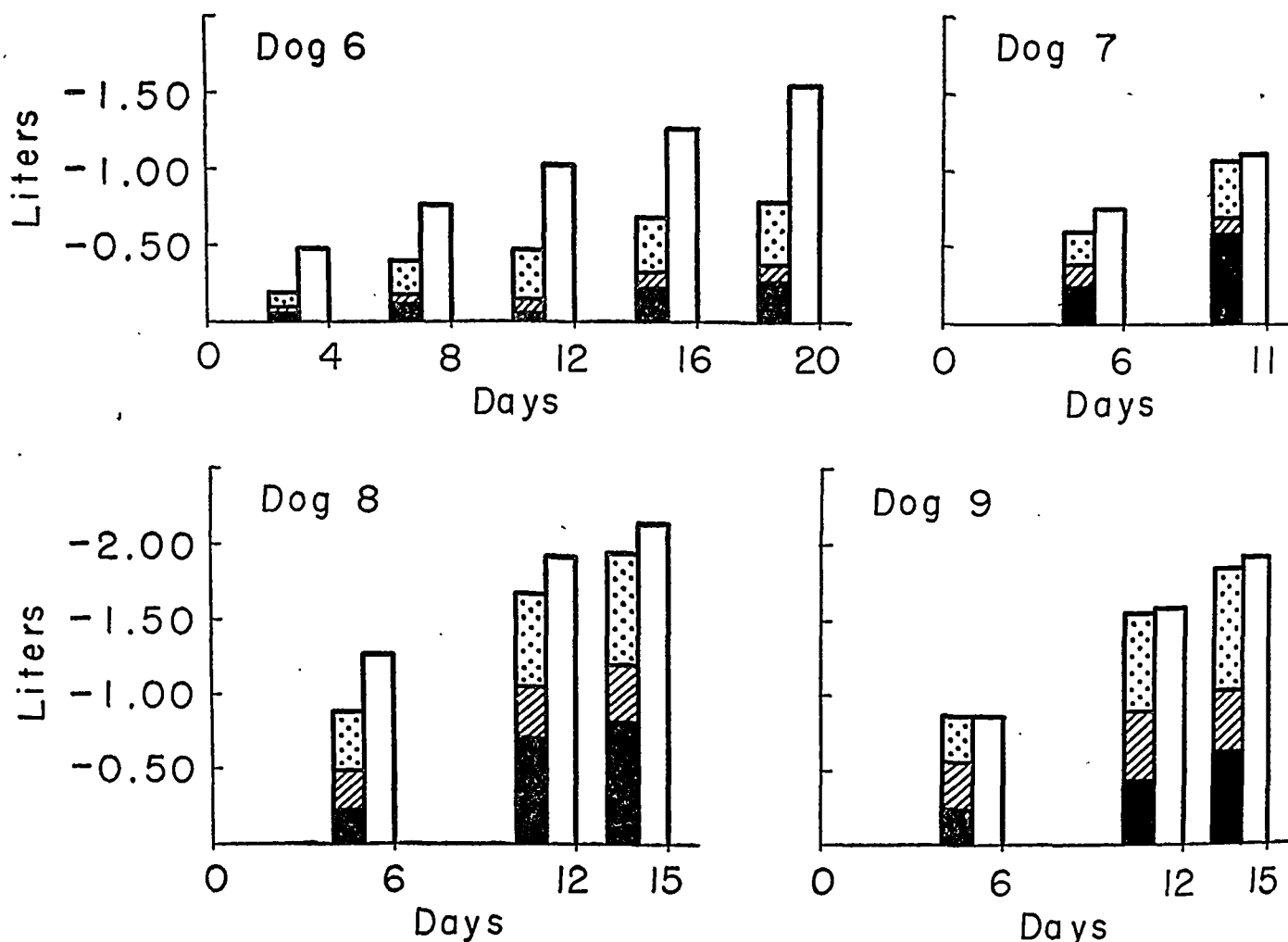


FIG. 1. DECREMENTS OF INTRACELLULAR WATER DURING WATER DEPRIVATION

Columns represent cell water loss cumulatively from the beginning of the experiment to the end of the day indicated. Decrements are represented as follows: water lost osmotically (ΔI_{AB}), solid black; water lost with K in excess of nitrogen ($\Delta I_{K'}$), cross-hatched; water lost with protein due to fasting (ΔI_P), dotted columns. Total cell water loss taken as the difference between total water and extracellular water losses (ΔI_T), blank columns.

sulfate over chloride and sodium, with a resultant rise in concentration of chloride and sodium in the serum, made available a fraction of cell water when water for excretion was greatly limited. The analogy of this experiment to the behavior of the animal deprived of water is clear.

The nature and extent of stimuli for the release of potassium in excess of nitrogen are unknown. Potassium is released from cells during hemorrhage (22). This condition shares with water deprivation the common factor of depleted extracellular volume, although anoxia may be an important factor. Evidence of a similar response under conditions lacking the teleological implications of our experiment has been produced by Gamble (23). He described an increased potassium excretion during sodium chlo-

ride ingestion by a patient who was not deprived of water. Stewart and Rourke (24) found a small increase in potassium excretion in patients receiving infusions of isotonic saline, which phenomenon they attributed to the preceding anesthesia and surgical trauma. A preliminary experiment in this laboratory (25) has revealed that the injection of hypertonic saline solution into a non-fasting dog not deprived of water is followed by a definite loss of potassium in excess of nitrogen. Such a loss of excess potassium did not occur following the injection of hypotonic saline solution or during a series of 24-hour control periods. Schwartz, Smith, and Winkler (21) have shown, in the acute experiment referred to above, that a large excretion of potassium took place during the 4 hours immediately following

the intravenous injection of hypertonic saline solution. The data of Darrow and Yannet (3) also show a consistent excess potassium loss following the administration of hypertonic saline solution to 4 dogs depleted of sodium. The precise nature of this phenomenon of potassium release awaits elucidation.

Significance. In water deprivation, the release of potassium mitigates, at the expense of cell fluid, not only the rise in total ionic concentration but also the depletion of extracellular volume. Perhaps the survival value of the sacrifice of cell water lies in the maintenance of an adequate circulation. Dill (26) found that the burro, an animal acclimatized to an environment of great heat, sweats almost pure water and thereby distributes the water loss over both phases of body

fluid. The concentration of salt in the sweat of Dill and his colleagues diminished as they became acclimatized to the heat of Boulder Dam. It is not known whether over a long period of time man can make this adjustment to the same degree as the burro. But the studies of Kerpel-Fronius (1) and of Nadal, Pedersen, and Maddock (2) have clearly shown that, in dehydration where sodium is also lost in large amounts, depletion of the extracellular phase alone leads to early diminution of plasma volume and consequent circulatory failure.

Heretofore, in studies of the effects of tropical and industrial heat, attention has centered on the loss and restoration of salt in the presence of ample water. The type of experiment reported here, in which lack of water is the main limiting

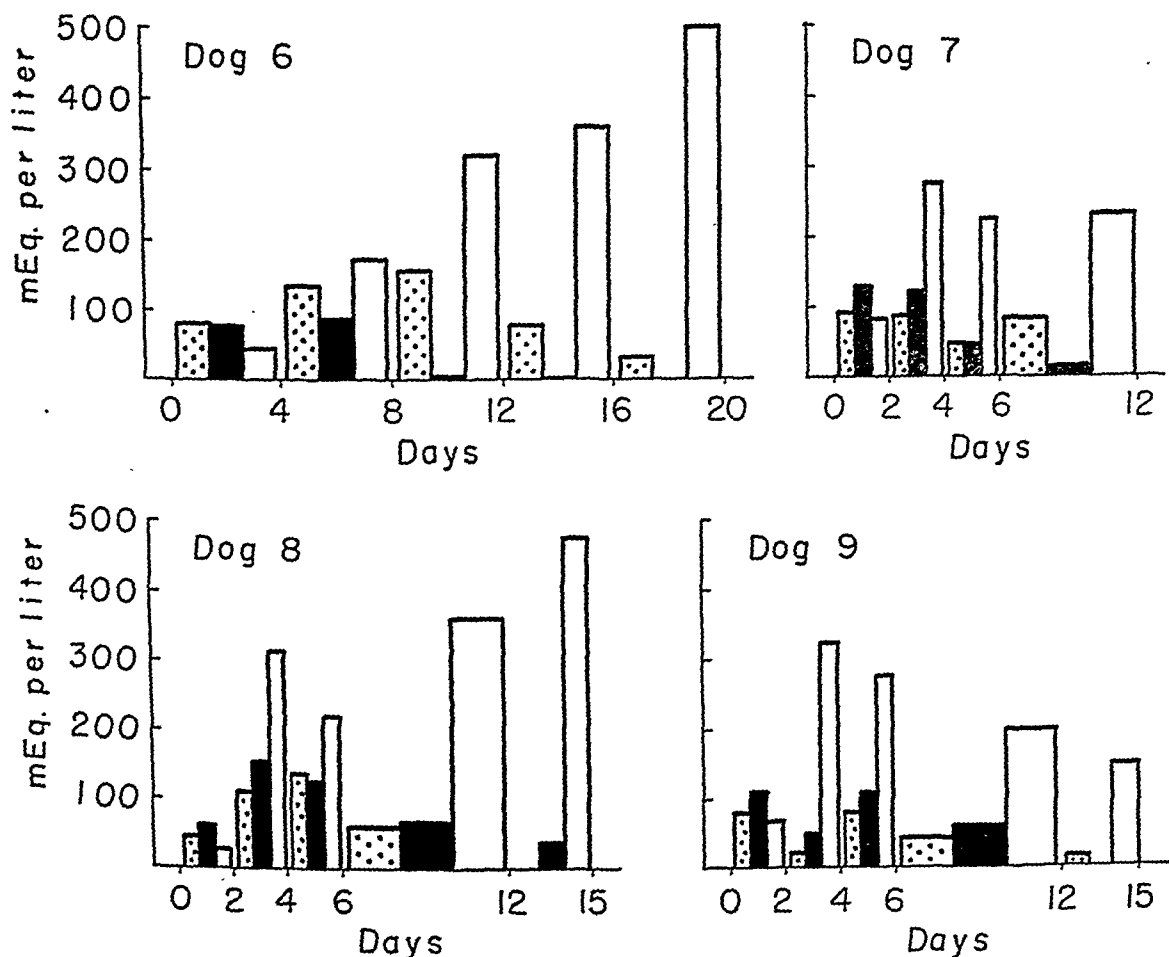


FIG. 2. CONCENTRATION OF ELECTROLYTES IN URINE

Columns represent concentration of electrolytes in urine for individual periods ending at the end of the day indicated, as follows: Cl, dotted; Na, solid black; K, blank columns.

condition, may assume more significance in the present emergency, where not infrequently circumstances arise in which water is at a premium.

SUMMARY

In 4 dogs deprived of water and food for periods of 11 to 20 days, the exchanges of salt and water were studied.

The concentration of sodium in serum rose, indicating hypertonicity of body fluids.

The total water loss greatly exceeded the extracellular water loss, indicating a substantial intracellular water loss.

The intracellular water loss was differentiated into three processes: water lost on an osmotic basis, water lost with cell destruction in fasting, and water lost with potassium released in excess of nitrogen.

The concentration of sodium and chloride in urine diminished, whereas that of potassium increased.

The significance of these physiological responses to the conditions imposed has been discussed.

The authors are indebted to Dr. John P. Peters for guidance in the planning of the experiment and in the interpretation of the results.

BIBLIOGRAPHY

- Kerpel-Fronius, E., Über die beziehungen zwischen salz-und wasserhaushalt bei experimentellen wasser-verlusten. *Ztschr. f. Kinderheilk.*, 1935, 57, 489.
- Nadal, J. W., Pedersen, S., and Maddock, W. G., A comparison between dehydration from salt loss and from water deprivation. *J. Clin. Invest.*, 1941, 20, 691.
- Darrow, D. C., and Yannet, H., Metabolic studies of the changes in body electrolyte and distribution of body water induced experimentally by deficit of extracellular electrolyte. *J. Clin. Invest.*, 1936, 15, 419.
- Hastings, A. B., and Eichelberger, L., The exchange of salt and water between muscle and blood. I. The effect of an increase in total body water produced by the intravenous injection of isotonic salt solutions. *J. Biol. Chem.*, 1937, 117, 73.
- Eichelberger, L., and Hastings, A. B., The exchange of salt and water between muscle and blood. III. The effect of dehydration. *J. Biol. Chem.*, 1937, 118, 205.
- Heppel, L. A., The electrolytes of muscle and liver in potassium-depleted rats. *Am. J. Physiol.*, 1939, 127, 385.
- Ferrebee, J. W., Parker, D., Carnes, W. H., Gerity, M. K., Atchley, D. W., and Loeb, R. F., Certain effects of desoxycorticosterone. The development of "diabetes insipidus" and the replacement of muscle potassium by sodium in normal dogs. *Am. J. Physiol.*, 1941, 135, 230.
- Hald, P. M., in Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Vol. II, Methods, p. 838, Williams and Wilkins, Baltimore, 1932.
- Hald P. M., The determination of the bases of serum and whole blood. *J. Biol. Chem.*, 1933, 103, 471.
- Crandall, L. A., Jr., and Anderson, M. X., Estimation of the state of hydration of the body by the amount of water available for the solution of sodium thiocyanate. *Am. J. Digest. Dis. and Nutrition*, 1934, 1, 126.
- Elkinton, J. R., and Taffel, M., The apparent volume of distribution of sulfocyanate and of sulfanilamide in the dog. *Am. J. Physiol.* (In press.)
- Harvey, S. C., The quantitative determination of the chlorids in the urine. *Arch. Int. Med.*, 1910, 6, 12.
- Butler, A. M., and Tuthill, E., An application of the uranyl zinc acetate method for determination of sodium in biological material. *J. Biol. Chem.*, 1931, 93, 171.
- Newburgh, L. H., Johnston, M. W., and Falcon-Lesses, M., Measurement of total water exchange. *J. Clin. Invest.*, 1929-30, 8, 161.
- Peters, J. P., Kydd, D. M., and Laviates, P. H., A note on the calculation of water exchange. *J. Clin. Invest.*, 1933, 12, 689.
- Anderson, R. J., and Lusk, G., Animal calorimetry. The interrelation between diet and body condition and the energy production during mechanical work. Thirteenth paper. *J. Biol. Chem.*, 1917, 32, 421.
- Laviates, P. H., The metabolic measurement of the water exchange. *J. Clin. Invest.*, 1935, 14, 57.
- Harrison, H. E., Darrow, D. C., and Yannet, H., The total electrolyte content of animals and its probable relation to the distribution of body water. *J. Biol. Chem.*, 1936, 113, 515.
- Darrow, D. C., Harrison, H. E., and Taffel, M., Tissue electrolytes in adrenal insufficiency. *J. Biol. Chem.*, 1939, 130, 487.
- Painter, E. E., Total body water in the dog. *Am. J. Physiol.*, 1940, 129, 744.
- Schwartz, B. M., Smith, P. K., and Winkler, A. W., The renal excretion of sulfate. *Am. J. Physiol.* (In press.)
- Stewart, J. D., and Rourke, G. M., Intracellular fluid loss in hemorrhage. *J. Clin. Invest.*, 1936, 15, 697.
- Gamble, J. L., Chemical anatomy, physiology, and pathology of extracellular fluid. A lecture syllabus. Department of Pediatrics, Harvard Medical School, Boston, Mass., 3rd Ed., 1941, Chart 34.
- Stewart, J. D., and Rourke, G. M., The effects of large intravenous infusions on body fluid. *J. Clin. Invest.*, 1942, 21, 197.
- Elkinton, J. R., Unpublished data.
- Dill, D. B., Life, Heat, and Altitude. Harvard Univ. Press, Cambridge, Mass., 1938.

INTUBATION STUDIES OF THE HUMAN SMALL INTESTINE. XXIII. A METHOD OF DETERMINING DIGESTIVE ACTIVITY IN ANY PORTION OF THE GASTRO-INTESTINAL TRACT, WITH SOME MEASUREMENTS OF PROTEIN DIGESTION IN THE STOMACH AND SMALL INTESTINE^{1, 2}

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Two essential physiological processes occur in the small intestine—the enzymatic cleavage of complex food substances into simpler nutritive compounds, and their transport through the intestinal wall into the body. Abnormality in one or both of these processes accounts in large part for the nutritional disturbance observed in many disorders, such as sprue, pancreatic disease, certain avitaminoses, regional enteritis, and ulcerative colitis. It is obviously desirable to have clinical methods of measuring each of these functions separately since a given disease may be due either to disturbance of digestion or of absorption, or both. The available tests of digestive function consist of “in vitro” determinations of the enzymatic activity of gastric or duodenal juice, and of the examination of the feces for undigested food material. Neither method is wholly satisfactory. The latter is relatively crude, it gives no quantitative data and the result is greatly influenced, not only by enzymatic activity, but also by the speed of passage of the intestinal contents. The former has the limitation of indicating only whether enzymes are present in the upper intestinal tract but does not disclose whether or not the conditions are favorable for their activity in the distal portions of the bowel.

It seemed to us that an improved method for studying digestion might be developed now that small intestinal intubation has become a standard procedure. We have therefore devised an apparatus, to be described presently, which utilizes this method and which makes it possible to test di-

rectly the digestion of suitable solid food materials, in any portion of the human gastrointestinal tract. The apparatus is so constructed that the test food stuff is exposed to the digestive enzymes only over a designated period and in that area of the bowel selected for study. The method has in our opinion certain points of superiority over those previously employed. Measurements can be made anywhere in the intestinal tract, the test object remains in a constantly renewed bath of intestinal juice while the products of enzyme reactions are carried away, thus avoiding their known retarding effect on farther enzyme activity. The method obviates the withdrawal of fluid for analysis and hence avoids the possible inactivation of enzymes which follows exposure to light, oxidation, and alterations in temperature and in pH. The present communication describes the apparatus and technique of its employment, and gives data on the digestion of protein in the normal and achlorhydric stomach and at various levels of the normal small intestine.

METHOD

The apparatus³ (Figure 1) consists of a cylinder of thin brass tubing (a), 4 cm. long and 1 cm. in outside diameter, the proximal end of which is narrowed and flanged for the attachment of a rubber tube (e). This cylinder serves as a housing for an inner brass cylinder (b) of equal length, which fits closely but may move freely within it. The inner cylinder is occluded at its proximal extremity by a piston-like disc, its walls are fenestrated in the distal 3 cm., and its exposed extremity is equipped with a screw cap (c) which permits the introduction of a test food substance weighing approximately 1 gram. By exerting air pressure through the rubber tube (e), the inner cylinder, wholly sheathed in

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² Aided by a grant from the Smith, Kline and French Laboratories, Philadelphia.

³ We are indebted to Mr. Carol Kelly for constructing the apparatus, as well as for valuable suggestions concerning its design.

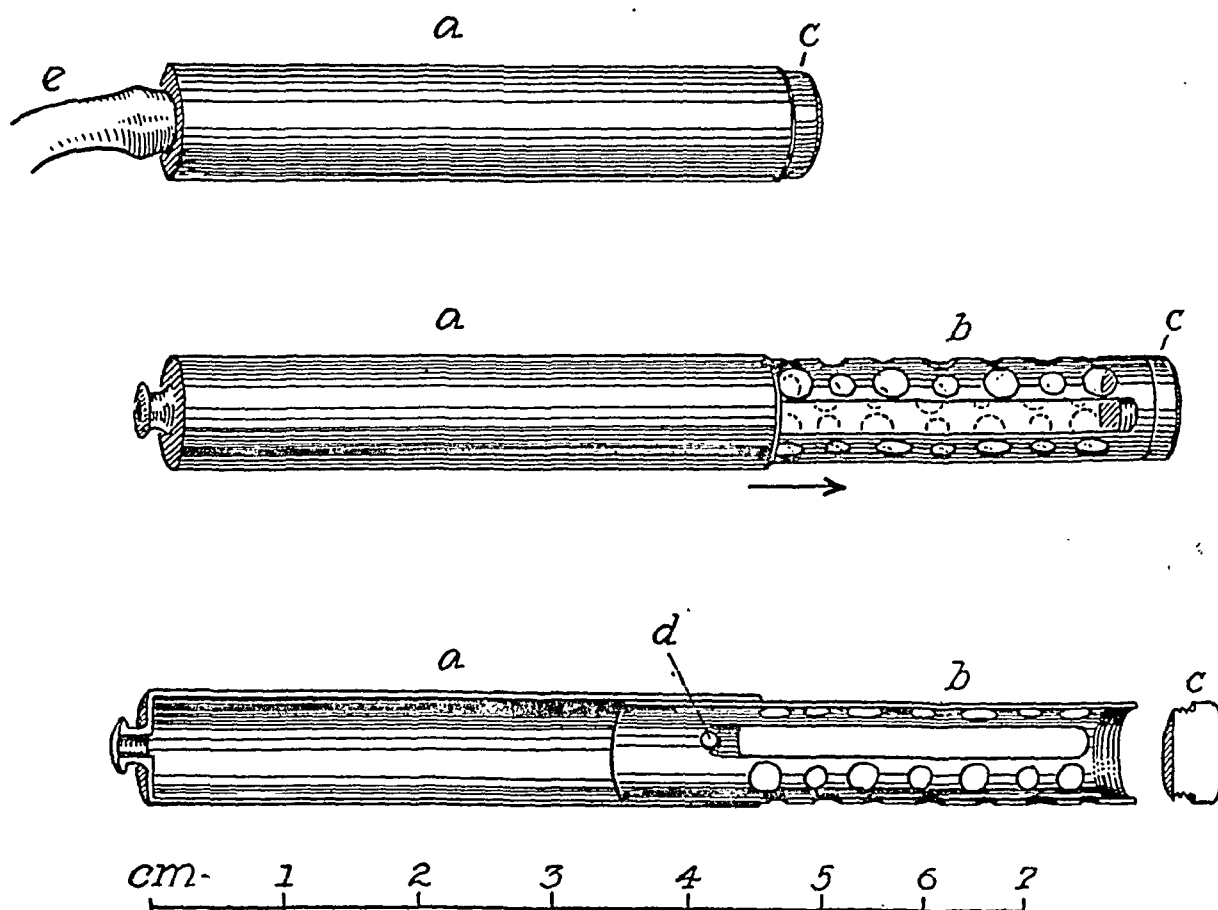


FIG. 1. THE APPARATUS FOR MEASURING THE DIGESTION OF A TEST FOOD SUBSTANCE

the housing, may be ejected so that its fenestrated distal portion protrudes from the open end of the housing, which contains in its wall a check pin (*d*) to prevent total expulsion of the inner cylinder. Sufficient friction is present between the two cylinders then to maintain the inner cylinder in its ejected position.

TECHNIQUE

Observations were begun in the morning, after an overnight fast. Depending on the portion of the intestinal tract to be studied, the apparatus described was attached either to a single lumen tube customarily used for duodenal intubation, or to one lumen of a Miller-Abbott tube, the other lumen of which was connected to an inflatable balloon. Into the inner cylinder of the apparatus was placed the carefully weighed test object and the cylinder was then retracted into the housing. The distal tip of the apparatus was dipped into melted paraffin which, when it cooled, sealed off the test object from contact with gastric or intestinal juice. With fluoroscopic guidance, the tube was then introduced to any portion of the intestinal tract, by the technique of intubation repeatedly described from this clinic. When the apparatus was in the desired position, the balloon was deflated and the inner cylinder ejected by means of air pressure. The apparatus then remained stationary, or advanced at most a few inches, permitting contact of the contained test material with the digestive enzymes. At the end of a measured period of time, the tube and

attached apparatus were withdrawn, either with the fenestrated cylinder in its ejected position or, if possible, retracted into the housing.

HISTORICAL

The ingestion of a food substance, usually in a fenestrated container, with subsequent observations of its condition after it had traversed the intestinal tract, is an experimental principle employed in the earliest demonstrations of digestive activity. Spallanzani (1), in studying digestion in a variety of animals, "... employed hollow globules of brass half an inch in diameter and pierced like a sieve, which I could open and shut at pleasure by means of a screw worked upon the edge of the two hemispheres into which each globule was divisible."

In an inaugural dissertation (Edinburgh, 1777) which is published as an appendix to Spallanzani's *Dissertations*, Stevens (2) describes observations in which he gave the subject of his experiments "a hollow silver sphere, divided into two cavities by a partition and perforated on the surface with a great number of holes, capable of admitting a needle: into one of these cavities was put four scruples and a half of raw beef, and into the other five scruples of raw bleak. The sphere was voided in twenty-one hours, when the beef was found to have lost one scruple and a half and the fish two scruples. . . . I procured another sphere with holes so large as to receive a crow's quill, and enclosed some beef a little masticated

into it. It was voided quite empty thirty-eight hours after it was swallowed."⁴

OBSERVATIONS ON PROTEIN DIGESTION

In the observations to be reported here, the described method was adapted for a study of protein digestion. Pork heart muscle was selected as a test object since it is easily obtainable in fresh condition after government inspection, is remarkably constant in protein content,⁵ is practically free of fat and fibrous tissue, and will not physically disintegrate and thus be lost from the fenestrated cylinder. For our purposes, raw muscle proved superior to dried or cooked muscle. The technique described above was employed in every detail. The test object was a columnar piece of heart muscle, weight approximately 0.8 gram.

The amount of digestion occurring after a 3-hour test was determined as follows: A portion of the heart muscle, immediately adjacent to the sample used in the capsule, was weighed and analyzed for nitrogen by a micro-Kjeldahl method. This permitted a calculation of the total nitrogen content of the sample employed in the test. At the conclusion of the observation, the nitrogen content of the tissue remaining in the capsule was determined, and the difference represented the amount of protein which had been digested. This is expressed as the percentage of that present in the original test object.

The gastric digestion of pork heart muscle was tested 13 times in 10 subjects without gastrointestinal disease and with a normal concentration of gastric acid. Ten subjects from the hospital wards or out-patient clinics, known to have true achlorhydria, were similarly tested. In all cases, the position of the tube in the stomach was determined fluoroscopically. Two professional subjects in good health were employed for the observations on the small intestine, of which 33 were made—11 in the duodenum, 13 in the jejunum, and 9 in the ileum.

⁴We can confirm this observation of Stevens'. On one occasion the apparatus became detached from the tube while in the stomach and was permitted to proceed through the intestinal tract. When recovered approximately thirty-six hours later it was empty.

⁵In 67 observations, the average protein content of pork heart muscle was 17 per cent, the maximum figure 19 per cent, and the minimum 16 per cent.

In order to determine the relationship between the time of exposure of the test object and the amount of digestion which occurred, measurements were made in the duodenum of a normal subject for periods of 30 to 240 minutes.

In order to discover whether the proteolytic action observed in a 3-hour test is due to the enzymes already present in the bowel, or depends upon the secretion of an additional supply, we have carried out, in a normal subject, experiments in which the pancreatic juice formed after the beginning of the observation was prevented from reaching the test object. This was accomplished (Figure 2) by the use of an inflated balloon (a) which occluded the lumen of the bowel, immediately proximal to which were perforations (b) through which constant suction was exerted by a Wangenstein apparatus, and 12 inches distal to the balloon was the digestion apparatus (c). The tube was introduced so that the balloon was in the distal duodenum or proximal jejunum. After obstructing the bowel by distending the balloon, continuous suction was applied withdrawing all contents from the bowel lumen proximal to it. The test substance was then exposed. This double procedure of occlusion and suction effectively

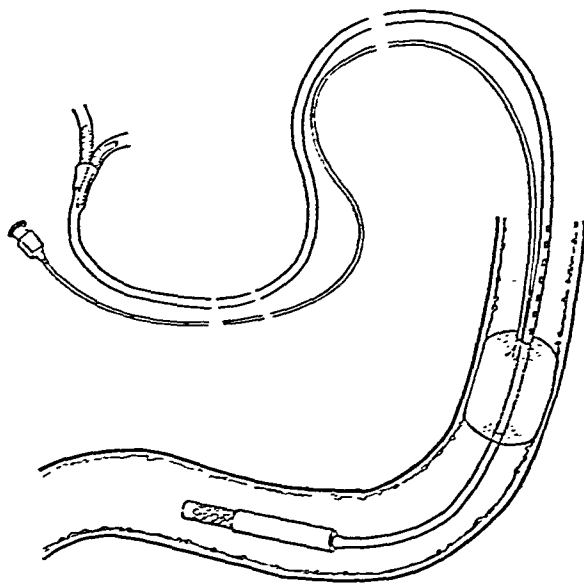


FIG. 2. THE ARRANGEMENT OF THE APPARATUS FOR OBSTRUCTING THE BOWEL PROXIMAL TO THE TEST OBJECT

Constant suction was applied through the perforations proximal to the occluding balloon.

prevents leakage of material past the balloon, as we have repeatedly demonstrated, using either a thin suspension of barium or a dilute solution of vital red.

In 3 observations, we attempted, by preliminary lavage, to remove the enzymes already present in the bowel before exposing the test substance. For this purpose, we used water, N/20 HCl, and dilute suspension of colloidal aluminum hydroxide.

RESULTS

Stomach

The measurements on the gastric digestion of protein in the normal and achlorhydric subjects are given in Figure 3. In the normal subjects, the average figure was 53 per cent, which ex-

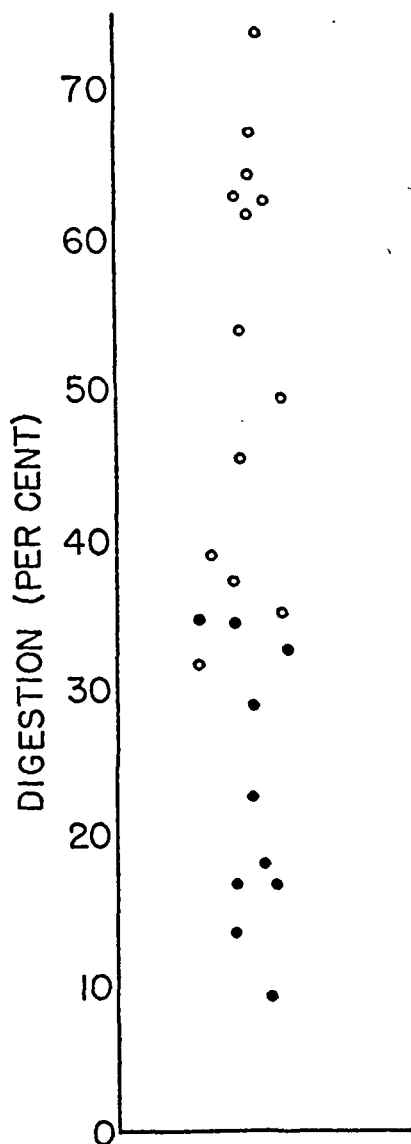


FIG. 3. THE GASTRIC DIGESTION OF PROTEIN IN NORMAL (OPEN CIRCLES) AND ACHLORHYDRIC (CLOSED CIRCLES) SUBJECTS

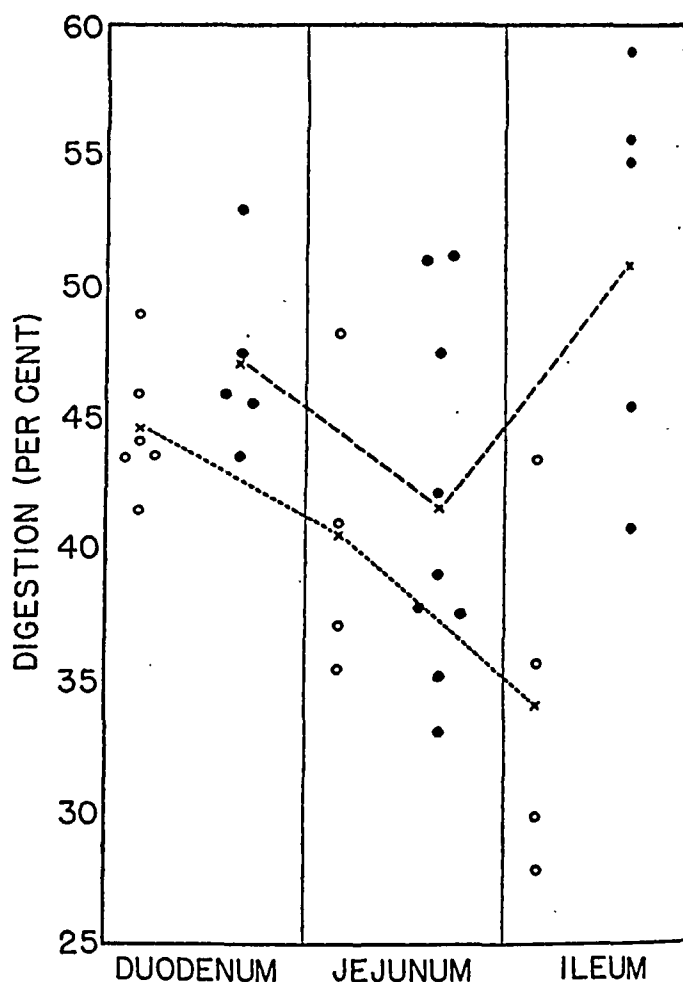


FIG. 4. THE DIGESTION OF PROTEIN AT DIFFERENT LEVELS OF THE SMALL INTESTINE IN 2 NORMAL SUBJECTS

Subject 1 (open circles), Subject 2 (closed circles). The broken lines join the average figures.

ceeds that for any other portion of the gastrointestinal tract. The individual variation was great, and in a single subject, tested 4 times, the results varied between 45 and 74 per cent. In the achlorhydric group, 1 subject had Addisonian anemia, with 16 per cent digestion. The others had no recognizable organic lesions of the stomach. The average figure was 23 per cent, the extremes were 9 and 35 per cent.

The results of the 33 observations made on 2 normal subjects, 1 female (Subject 1) and 1 male (Subject 2), are shown in Figure 4. Each point represents a single observation, made in that portion of the small intestine indicated in the figure. The points joined by lines show the averages for both subjects. It will be observed that the most constant results were obtained in the duodenum (average 45 per cent), while greater variation occurred in the jejunum (average 41 per cent)

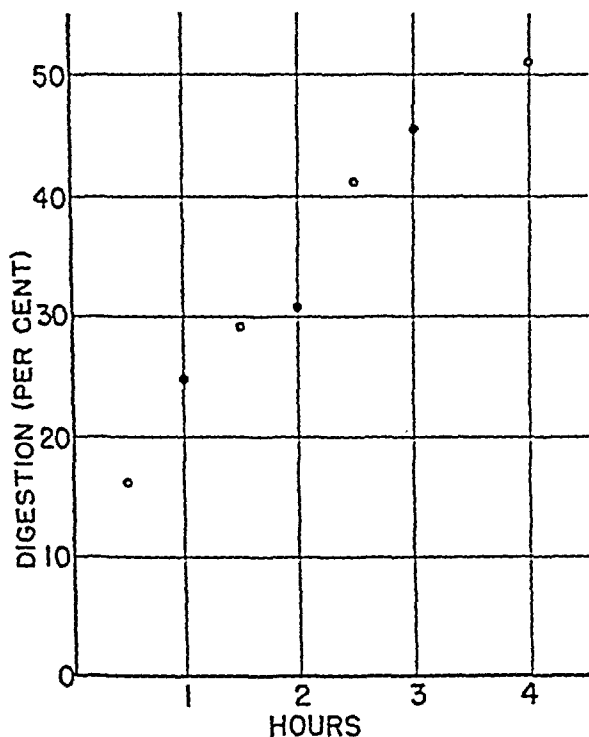


FIG. 5. THE RELATIONSHIP BETWEEN THE TIME OF EXPOSURE AND THE DIGESTION OF PROTEIN IN THE DUODENUM OF A NORMAL SUBJECT

and ileum (average 42 per cent). The average readings for the 2 subjects were almost identical, both in the duodenum and in the jejunum. In the ileum, however, the results in the 2 subjects differed widely; Subject 1 digested, on the average, 31 per cent of the test protein, while Subject 2 digested 50 per cent.

The results of testing the digestion of heart muscle at half-hourly intervals up to 4 hours are shown in Figure 5. The readings so obtained arrange themselves along a parabolic curve, and when their logarithms are plotted, a straight line results. This type of curve characterizes the general reaction of a single enzyme and substrate.

The exclusion of digestive juice from the test object

In one observation, digestion was measured in the upper jejunum. No effort was made to remove the enzymes already present, but all intestinal content proximal to the area studied was withdrawn by constant suction during the period of observation. Forty-four per cent of the test object was digested in 3 hours. This is essentially

the figure which had been obtained on previous tests in this exact location, when the normal current of intestinal juice had not been diverted. In 3 observations, efforts were made, by careful preliminary lavage, to remove or inactivate all enzymes present in the segment of bowel to be studied. Water, N/20 HCl, and dilute suspension of an adsorbant, colloidal aluminum hydroxide, were each used once, for periods of lavage from one-half to one hour, in amounts from 250 to 800 cc. This procedure reduced the digestion only moderately, to 29, 28, and 36 per cent respectively. This is in agreement with the observations of Owles (3), who found that prolonged washing of the jejunum was required to remove previously secreted erepsin.

DISCUSSION

It may be objected that, in this method of measuring digestive activity, the presence of the tube and metal apparatus produces mechanical irritation, which is known (3) to influence the secretion of enzymes. This possibility cannot be ignored, but it appears to be a difficulty inherent in any method now known. The method gives no information concerning the extent to which the substance being studied is broken down by enzymatic activity. In the observations on protein digestion, described here, the ultimate degree of proteolysis is undetermined. When the enzyme activity at the surface of the test object has produced a soluble protein component, that material is lost from the test object, regardless of the degree of digestion which has occurred. For example, the test object might be equally reduced in weight by the formation, in one case, of soluble acid metaprotein, formed by the action of gastric HCl, and in another, by the solution of products of tryptic activity in the duodenum. The test would indicate an equal amount of digestion, although, in the latter case, the cleavage of the protein molecule would be much greater. However, if its inherent limitations are taken into proper account in the interpretation of the results, the method appears to provide a useful and simple procedure for measuring digestion in portions of the human intestinal tract which, until recently, have been sufficiently inaccessible to escape careful study.

The general method has thus far been adapted only to a study of protein digestion. Suitable test

objects to measure the carbohydrate and fat splitting power of the digestive juice can undoubtedly be developed, and, it is hoped, a satisfactory test substance containing all three major foodstuffs may be found, thus obviating the limitation of testing only one substance at a time.

The results of the study of protein digestion in the stomach are in accord with many earlier observations, the most familiar of which are those of Beaumont (4). They indicate that a very considerable proteolytic cleavage can occur in this organ. The conditions of this test, in which meat alone is introduced, are, of course, somewhat unusual. The recent observations of Beazell (5) show that when a protein and carbohydrate meal is ingested, gastric digestion of carbohydrate is considerable, while that of protein is negligible. However, the conditions are so different in the two types of experiments that the results are not necessarily conflicting. The relative incapacity of the achlorhydric stomach to digest protein is probably due to deficiency both in acid and in enzyme secretion, for it is known (6) that in this condition the peptic, as well as acid, secretion is often deficient, and even though it were not, the pH of the achlorhydric stomach is not optimal for peptic activity.

The digestion of protein which, as we have shown, can begin in the ileum is presumably due to the presence of pancreatic trypsin which has been carried in active form into the distal bowel. This must be true, since it is known that the only other proteolytic enzyme there, the erepsin of the succus entericus, does not attack native proteins, but acts only on polypeptides and dipeptides. The further fact appears that a sufficient quantity of protein-splitting enzymes is present throughout the gut in the fasting state, so that even though additional amounts are prevented from reaching the distal bowel during the period of study, a very considerable breakdown of protein occurs. The observation, therefore, that the digestion of protein can begin in the distal segments of the bowel, without any preliminary proteolysis in the upper intestine, and the demonstration that this digestion can be carried out solely by the enzymes already present, even though the subject has fasted for 18 hours, emphasizes the fact that the normal digestive tract possesses a very impressive margin of safety with respect to the breakdown of protein

foods. These observations aid in explaining the sometimes remarkably complete digestion which occurs in clinical cases where large segments of the small bowel have been resected, where marked small intestinal hypermotility exists, or where entero-enteric fistulae have shortened the course taken by the intestinal contents.

SUMMARY

A method has been developed for measuring digestive activity at any level of the human gastrointestinal tract. A specially devised apparatus containing a test food substance is introduced into the intestine by intubation. When it has reached the desired position, the test substance is exposed to the action of the intestinal juice. The digestion which occurs in a measured time can be determined by appropriate analysis of the remaining portion of the test substance. We have adapted the method to a study of protein digestion, using pork heart muscle as the test material. The results indicate that substantial proteolytic activity occurs in the normal stomach, far less in the achlorhydric stomach. Throughout the normal small intestine, the concentration of proteolytic enzymes is sufficiently high to effect a very considerable amount of digestion, regardless of the point at which the process begins, or whether a further supply of enzymes is available during the period of the test. The method should prove useful in studying clinical problems which involve abnormalities in digestion.

We are indebted to Dr. Walter G. Karr for many helpful suggestions, and to Miss Thelma Cline, R.N., for assistance in the intubation.

BIBLIOGRAPHY

1. Spallanzani, Abbé Lazarus, *Dissertations Relative to the Natural History of Animals and Vegetables*. London, 1799, Vol. I.
2. *Ibid.* Appendix, p. 375.
3. Owles, W. H.: *Investigations of the functions of the small intestine in man by intestinal intubation*. Clin. Sc., 1937, 3, 21.
4. Beaumont, William, *Experiments and Observations on the Gastric Juice*. Plattsburgh, 1833.
5. Beazell, J. M., *A re-examination of the role of the stomach in the digestion of carbohydrate and protein*. Am. J. Physiol., 1941, 132, 42.
6. Rasmussen, R. A., and Brunschwig, A., *Peptic activity of achlorhydric human gastric juices from carcinomatous stomachs. A comparative study*. Proc. Soc. Exper. Biol. and Med., 1941, 46, 298.

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objects to measure the carbohydrate and fat splitting power of the digestive juice can undoubtedly be developed, and, it is hoped, a satisfactory test substance containing all three major foodstuffs may be found, thus obviating the limitation of testing only one substance at a time.

The results of the study of protein digestion in the stomach are in accord with many earlier observations, the most familiar of which are those of Beaumont (4). They indicate that a very considerable proteolytic cleavage can occur in this organ. The conditions of this test, in which meat alone is introduced, are, of course, somewhat unusual. The recent observations of Beazell (5) show that when a protein and carbohydrate meal is ingested, gastric digestion of carbohydrate is considerable, while that of protein is negligible. However, the conditions are so different in the two types of experiments that the results are not necessarily conflicting. The relative incapacity of the achlorhydric stomach to digest protein is probably due to deficiency both in acid and in enzyme secretion, for it is known (6) that in this condition the peptic, as well as acid, secretion is often deficient, and even though it were not, the pH of the achlorhydric stomach is not optimal for peptic activity.

The digestion of protein which, as we have shown, can begin in the ileum is presumably due to the presence of pancreatic trypsin which has been carried in active form into the distal bowel. This must be true, since it is known that the only other proteolytic enzyme there, the erepsin of the succus entericus, does not attack native proteins, but acts only on polypeptides and dipeptides. The further fact appears that a sufficient quantity of protein-splitting enzymes is present throughout the gut in the fasting state, so that even though additional amounts are prevented from reaching the distal bowel during the period of study, a very considerable breakdown of protein occurs. The observation, therefore, that the digestion of protein can begin in the distal segments of the bowel, without any preliminary proteolysis in the upper intestine, and the demonstration that this digestion can be carried out solely by the enzymes already present, even though the subject has fasted for 18 hours, emphasizes the fact that the normal digestive tract possesses a very impressive margin of safety with respect to the breakdown of protein

foods. These observations aid in explaining the sometimes remarkably complete digestion which occurs in clinical cases where large segments of the small bowel have been resected, where marked small intestinal hypermotility exists, or where entero-enteric fistulae have shortened the course taken by the intestinal contents.

SUMMARY

A method has been developed for measuring digestive activity at any level of the human gastrointestinal tract. A specially devised apparatus containing a test food substance is introduced into the intestine by intubation. When it has reached the desired position, the test substance is exposed to the action of the intestinal juice. The digestion which occurs in a measured time can be determined by appropriate analysis of the remaining portion of the test substance. We have adapted the method to a study of protein digestion, using pork heart muscle as the test material. The results indicate that substantial proteolytic activity occurs in the normal stomach, far less in the achlorhydric stomach. Throughout the normal small intestine, the concentration of proteolytic enzymes is sufficiently high to effect a very considerable amount of digestion, regardless of the point at which the process begins, or whether a further supply of enzymes is available during the period of the test. The method should prove useful in studying clinical problems which involve abnormalities in digestion.

We are indebted to Dr. Walter G. Karr for many helpful suggestions, and to Miss Thelma Cline, R.N., for assistance in the intubation.

BIBLIOGRAPHY

1. Spallanzani, Abbé Lazarus, *Dissertations Relative to the Natural History of Animals and Vegetables*. London, 1799, Vol. I.
2. *Ibid.* Appendix, p. 375.
3. Owles, W. H.: *Investigations of the functions of the small intestine in man by intestinal intubation*. Clin. Sc., 1937, 3, 21.
4. Beaumont, William, *Experiments and Observations on the Gastric Juice*. Plattsburgh, 1833.
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6. Rasmussen, R. A., and Brunschwig, A., Peptic activity of achlorhydric human gastric juices from carcinomatous stomachs. A comparative study. *Proc. Soc. Exper. Biol. and Med.*, 1941, 46, 298.

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